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Gene transcription mediated local adaptation of Babine Lake tributary rainbow trout

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GENE TRANSCRIPTION MEDIATED LOCAL ADAPTATION OF BABINE LAKE TRIBUTARY RAINBOW TROUT

by

Kyle W. Wellband

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DECLARATION OF CO-AUTHORSHIP

I. Co-Authorship Declaration

I hereby declare that this thesis incorporates material that is result of joint research, as follows:
This thesis also incorporates the outcome of a joint research undertaken in collaboration with and under the supervision of professor Dr. Daniel Heath. The collaboration is covered in Chapter 2 of the thesis. In all cases, the key ideas, primary contributions, experimental designs, data analysis and interpretation, were performed by the author, and the contribution of co-authors was solely in an advisory capacity.

I am aware of the University of Windsor Senate Policy on Authorship and I certify that I have properly acknowledged the contribution of other researchers to my thesis, and have obtained written permission from each of the co-author(s) to include the above material(s) in my thesis.

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II. Declaration of Previous Publication

This thesis includes one original paper that has been previously published/submitted for publication in peer reviewed journals, as follows:

Thesis Chapter	Publication title/full citation	Publication status*
Chapter 2	Environmental associations with gene transcription in Babine Lake rainbow trout: evidence for local adaptation.	Submitted to Molecular Ecology, June 7, 2012

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ABSTRACT

The adaptation of populations to their local environments has implications for speciation theory as well as the conservation and management of genetic diversity in those populations. The genetic mechanisms that underlie the process of local adaptation remain poorly characterized; however recent evidence suggests a role for the evolution of gene transcription regulation in the development of local adaptations. The goal of this thesis is to examine transcriptional divergence among genetically structured populations of juvenile rainbow trout from Babine Lake, BC and test the hypothesis that transcriptional divergence in this system reflects local adaptation. This thesis provides evidence that transcriptional divergence is present among Babine Lake tributaries, that gene transcription correlates with specific environmental parameters of tributaries and that patterns of divergence do not reflect a pattern of evolution by neutral drift. These results reinforce the need to conserve salmonid populations at fine spatial scales to preserve functional (transcriptional) genetic diversity.

DEDICATION

To SKDM

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CHAPTER I

GENERAL INTRODUCTION

The process of local adaptation underpins both theories of speciation (e.g. Schluter 2001) and the concept of evolutionary significant units in species conservation and management (Fraser and Bernatchez 2001). The idea that natural selection shapes the evolution of populations and species is not new (Darwin 1859). However, it is only recently that natural selection has found resurgence in speciation theory where it first drives divergence among groups that is then followed by the development of genetic incompatibilities solidifying the diverged groups as incipient species (Schluter 2001). The relevance of local adaptations to management and conservation of species should not be overlooked either. Despite the wide use of neutral markers to define conservation units, the rationale behind the conservation of genetic diversity is to maximize and maintain levels of genetic diversity for functional traits presumed to be locally adapted (Fraser and Bernatchez 2001).

Local adaptation

Local adaptations are an extension of evolutionary adaptation where populations evolve via natural selection to be more fit in their local habitat than in any other habitat in which they could exist. While local adaptations can form in a variety of situations, conditions that frequently promote local adaptations include limited gene flow and environmentally heterogeneous habitats across the species' distribution (Kaweki and Ebert 2004). In salmonid fish, local adaptations have been the focus of a large body of research investigating the observed diversity of life histories in this group (see reviews by

Taylor 1991, Garcia de Leaniz et al. 2007). Salmonid species' distributions cover large portions of the northern hemisphere, meaning individuals and populations have the potential to experience a wide variety of environmental conditions. The specific homing behavior of salmonids coupled with high levels of natal philopatry results in the formation of local populations with reduced gene flow (Quinn 2005). As such, the conditions favoring local adaptations are believed to be present in many salmonid species. Despite the considerable body of literature regarding local adaptations in salmonids, there is a lack of consensus about the extent and scale of local adaptions for salmonids (Fraser et al. 2011). In a meta-analysis, Fraser et al. (2011) found the extent and scale of local adaptations to be trait and context dependent owing to the complex interactions of selection, drift and gene flow for any given set of populations and selective forces.

Fitness variables

Juvenile salmonids experience high levels of mortality in their first year, indicating this is likely a period of strong selection for fitness related traits. Phenotypic traits related to survival and successful reproduction are obviously tightly bound to individuals' fitness. While salmonids invariably experience both soft (density-dependent) and hard (density-independent) modes of selection (Young 2004), selection associated with survival traits likely falls on the harder side of the spectrum. Reviews of local adaptation (Taylor 1991, Garcia de Leaniz et al. 2007) have highlighted the roles of temperature and pathogens as selective agents shaping local adaptations.

Groups of genes demonstrated to be differentially expressed among life histories and populations hint at the role of metabolic stress and thermal regime in driving divergent selection (e.g. Whitehead and Crawford 2006, St-Cyr et al. 2008). The response to metabolic stress is activated by the hypothalymus-pituitary-interrenal axis that releases cortisol into the blood stream (Mommsen et al. 1999). Cortisol binds to glucocorticoid receptors activating a signaling pathway that ultimately results in modulation of growth, metabolic and immune related gene transcription (Aluru and Vijayan 2009). The modulation of gene transcription for these traits is believed to be adaptive and may be a substrate for local adaptation. Furthermore, gene transcription mediated local adaptation to temperature regime has been demonstrated for *Fundulus heteroclitus* (Whitehead and Crawford 2006). In addition to metabolic stress, the role of pathogen-mediated selection on the immune system is well established (Sommer 2005). Much of this evidence for salmonids comes from studies of major histocompatibility genes (e.g. Dionne et al. 2009, Evans and Neff 2009, de Eyto et al. 2011) though selection has been inferred for other immune related loci (Tontori et al. 2010).

Gene transcription evolution

In contrast to the volume of knowledge regarding adaptations of phenotype to local environments, comparatively little is know about the molecular genetic mechanisms that underlie most local adaptations (Fraser et al. 2011). A promising approach to the study of molecular genetic mechanisms of local adaptation is the use of transcriptomics. Following the 'central dogma' of molecular biology, transcription of messenger RNA from gene coding DNA is one step in the expression of phenotype. Regulation of

transcription is controlled by often complex interactions of proteins such as transcriptional activators, repressors and enhancers with promoter binding regions in the DNA sequence upstream of genes as well as the RNA polymerase itself (Ptashne and Gann 1997). In addition, epigenetic effects including methylation and histone modification have been implicated in transcriptional regulation (e.g. Grewal and Moazed 2003). Despite additional layers of complexity during post-transcriptional regulation of gene expression, transcript levels are generally correlated with protein levels (Schwanhausser et al. 2011).

The heritability of gene transcription is believed to be largely non-additive (Gibson and Weir 2005) and there is evidence for non-additive genetic effects in transcriptional traits of several species of salmon (e.g. Normandeau et al. 2009, Aykanat et al. 2012). Rapid evolution of gene transcription has been demonstrated in laboratory experiments (Rifkin et al. 2005), natural populations (Aykanat et al. 2011) and in response to domestication (Roberge et al. 2006) suggesting that evolution of gene transcription only requires a couple of generations. Given its capability for rapid evolution and the level of control it exerts on phenotype, transcription holds promise for explaining some of the molecular mechanisms associated with local adaptation. The evolution of gene transcription is primarily governed by stabilizing selection (Gilad et al. 2006); however, parallel evolution of certain transcriptional patterns associated with growth and survival has been demonstrated (St-Cyr et al. 2008, Jeukins et al. 2010) suggesting a role for environmentally-mediated divergent selection in optimizing gene transcription among life histories.

The role of transcription in regulating responses to metabolic stress (Wiseman et al. 2007) and in response to immune challenge (Raida and Buchmann 2008) suggests there is a continuum of transcription states that have the potential to be under selection. There are four possible outcomes that differ with the combination of directional selection on resting state and response to a challenge. The first possibility is that directional selection acts on resting state transcription but not the transcriptional response to challenge resulting in a reaction norm similar to Figure 1.1A. Directional selection may act only on the response (Figure 1.1B) or it may act on both the resting state transcription as well as transcriptional response (Figure 1.1C). Stabilizing selection may be acting on gene transcription (Gilad et al. 2006) and would result in similar resting states and responses of gene transcription among groups (not shown). Finally, there is the possibility that differences among populations are the result of evolution by genetic drift (also not shown) due to the population structure and small effective population sizes present for salmonids (e.g. Heath et al. 2002).

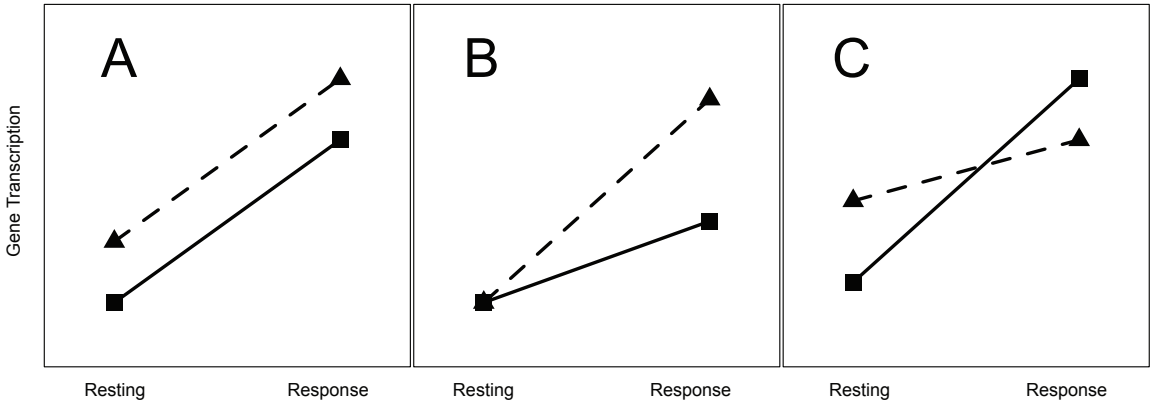

Figure 1.1: Outcomes of differential selection on resting and response levels of transcription of a single gene. Continuous and dashed lines represent different populations. Selection on resting state transcription but not response (A), selection on response levels alone (B) and selection on both resting state and response of gene transcription (C).

Molecular genetic tools such as microarrays and real-time quantitative polymerase chain reaction (qPCR) have been developed that allow the amount of messenger RNA (mRNA) in tissue to be quantified. The relative instability of mRNAs require that they be reverse-transcribed into a comparatively more stable molecule, complementary DNA (cDNA), while maintaining the genetic information contained with its sequence. Quantification of cDNA for a specific gene makes use of DNA hybridization technology, either using a gene specific probe (microarray) or gene-specific primers (q-RT-PCR), and a reporter dye to measure accumulation of cDNA for a specific gene. In q-RT-PCR, cDNA is amplified using gene-specific primers and the accumulation of PCR product is measured in real-time (cycle by cycle) with the use of fluorescent dyes. The quantity of starting material in a q-RT-PCR assay can then be calculated using accumulation of fluorescence and the dynamics of a PCR reaction (Tuomi et al. 2010). Microarray technology involves fluorescently labeling cDNA and hybridizing it to gene specific probes that are fixed to a glass slide. Slides are then scanned and the amount of fluorescence measured is proportional to the amount of cDNA hybridized to each gene specific site. Microarrays provide simultaneous quantification of transcription at hundreds or even thousands of genes but come with increased costs associated with appropriately replicated experimental designs to allow sufficient power to detect small effects. q-RT-PCR assays on the other hand have incredible sensitivity but suffer from the need to choose a limited set of candidate genes and the need to standardize to account for different amounts of starting material in individual assays. Next-generation sequencing technologies have recently been adapted for the study of

transcriptomics and hold great promise for future studies of gene expression (e.g. Jeukins et al. 2010).

Babine Lake rainbow trout

Babine Lake is a large freshwater lake in central British Columbia. The lake has a watershed area of 450 Km^2 and is one of the major drainages of the Skeena River system. Babine Lake supports First Nation's and recreational fishing opportunities in addition to being an important nursery for Sockeye salmon, *Oncorhynchus nerka* (Bustard 1989). Resident rainbow trout (*Oncorhynchus mykiss* Walbaum) comprise ~70% of the recreational sport fishery on the lake; however, declines have been noted since the early 1980s (Bustard 1989, 1990). Rainbow trout spawn in the lake's tributary streams during late-May and June (Bustard 1990) and rear in the tributary streams for at least three years before migrating to the lake to spend their lives as adults (Bustard 1989). Tributary environments vary from small and sometimes ephemeral streams to large rivers with much more consistent conditions. Rainbow trout populations rearing across the variety of habitats surrounding Babine Lake are genetically structured indicating reduced gene flow among populations (Koehler 2010). As a result, the conditions promoting the development of local adaptions are present for Babine Lake juvenile rainbow trout populations rearing in tributaries.

Thesis objectives

This thesis investigates gene transcription mediated local adaptation among genetically structured populations of rainbow trout from Babine Lake. It compares

transcriptional differences among populations and discusses the influence of environment as a driver of selection for these traits as well as selection in the face of gene flow and genetic drift. Throughout it considers transcriptional adaptation in two contexts: 1) transcription at resting state and 2) transcriptional response to immune and metabolic challenges, as both are potentially selected for.

Chapter 2 utilizes a candidate gene approach to explore transcriptional differences among populations for a set of functionally important metabolic and immune genes. Transcriptional differences among populations are demonstrated and then correlated with environmental variables to provide corroborating evidence that habitat variability is driving transcriptional divergence among populations.

Chapter 3 compares genetic divergence among populations at a suite of functionally relevant genes using microarray technology. Functional divergence is then compared with neutral divergence estimated using microsatellites. Comparisons of differentially expressed genes and those under different modes of selection are discussed in the context of gene flow and drift.

These chapters address whether transcriptional divergence exists for juvenile rainbow trout populations rearing in Babine Lake tributaries and dispute genetic drift as the cause of this divergence. I argue that natural selection is the cause of the observed transcriptional divergence and that this divergence represents local adaptation to tributary environments. These results reinforce the evolution of gene transcription regulation as a genetic mechanism of local adaptation and highlight the need to conserve local populations to maintain high levels of genetic diversity and adaptive potential.

References

Aluru N, Vijayan MM (2009) Stress transcriptomics in fish: A role for genomic cortisol signaling. General and Comparative Endocrinology, **164**, 142-150.

Aykanat T, Heath JW, Heath DD (2012) Additive, non-additive and maternal effects of cytokine transcription in response to immunostimulation with Vibrio vaccine in Chinook salmon (*Oncorhynchus tshawytscha*). Immunogenetics, IN PRESS.

Aykanat T, Thrower FP, Heath DD (2011) Rapid evolution of osmoregulatory function by modification of gene transcription in steelhead trout. Genetica, **139**, 233-242.

Bustard D (1989) Assessment of rainbow trout recruitment from streams tributary to Babine Lake. Consultant report prepared for Skeena Region Fisheries Branch, BC Ministry of Environment and Parks. Smithers, BC. 1-71.

Bustard D (1990) Sutherland River rainbow trout radio telemetry studies 1989. Consultant report prepared for Skeena Region Fisheries Branch, BC Ministry of Environment. Smithers, BC. 1-56.

Darwin C (1859). On the origin of species. John Murray, London.

de Eyto E, McGinnity P, Huisman J, Coughlan J, Consuegra S, Farrell K, O'Toole C, Tufto J, Megens HJ, Jordan W, Cross T, Stet RJM (2011) Varying disease-mediated selection at different life-history stages of Atlantic salmon in fresh water. Evolutionary Applications, **4**, 749-762.

Dionne M, Miller KM, Dodson JJ, Bernatchez L (2009) MHC standing genetic variation and pathogen resistance in wild Atlantic salmon. Philosophical Transactions of the Royal Society B, **364**, 1555-1565.

Evans ML, Neff BD (2009) Major histocompatibility complex heterozygote advantage and widespread bacterial infections in populations of Chinook salmon (*Oncorhynchus tshawytscha*). Molecular Ecology, **18**, 4716-4729.

Fraser DJ, Bernatchez L (2001) Adaptive evolutionary conservation: towards a unified concept for defining conservation units. Molecular Ecology, **10**, 2741-2752.

Fraser DJ, Weir LK, Bernatchez L, Hansen MM, Taylor EB (2011) Extent and scale of local adaptation in salmonid fishes: review and meta-analysis. Heredity, **106**, 404-420.

Garcia de Leaniz C, Fleming IA, Einum S, Verspoor E, Jordan WC, Consuegra S, Aubin-Horth N, Lajus D, Letcher H, Youngson AF, Webb JH, Vøllestad LA, Villanueva B, Ferguson A, Quinn TP (2007) A critical review of adaptive genetic variation in Atlantic salmon: implications for conservation. Biological Reviews, **82**, 173-211.

Gibson G, Weir B (2005) The quantitative genetics of transcription. Trends in Genetics, **21**, 616-623.

Gilad Y, Oshlack A, Rifkin SA (2006) Natural selection on gene expression. Trends in Ecology and Evolution, **22**, 456-461.

Grewal SIS, Moazed D (2003) Heterochromatin and epigenetic control of gene expression. Science, **301**, 798-802.

Heath DD, Busch C, Kelly J, Atagi DY (2002) Temporal change in genetic structure and effective population size in steelhead trout (*Oncorhynchus mykiss*). Molecular Ecology, **11**, 197-214.

Jeukins J, Renault S, St-Cyr J, Nolte AW, Bernatchez L (2010) The transcriptomics of sympatric dwarf and normal lake whitefish (*Coregonus clupeaformis* spp., Salmonidae) divergence as revealed by next-generation sequencing. Molecular Ecology, **19**, 5389-5403.

Kawecki TJ, Ebert D (2004) Conceptual issues in local adaptation. Ecology Letters, **7**, 1225-1241.

Koehler RA (2010) Adult distribution and the effects of dispersal and genetic drift on population genetic structure of resident rainbow trout (*Oncorhynchus mykiss*) in Babine Lake, British Columbia. M.Sc. Thesis, University of Windsor. 1-89.

Mommsen TP, Vijayan MM, Moon TW (1999) Cortisol in teleost: dynamics, mechanisms of action, and metabolic regulation. Reviews in Fish Biology and Fisheries, **9**, 211-268.

Normandeau E, Hutchings JA, Fraser DJ, Bernatchez L (2009) Population-specific gene expression responses to hybridization between farm and wild Atlantic salmon. Evolutionary Applications, **2**, 489-503.

Podrabsky JE, Somero GN (2004) Changes in gene expression associated with acclimation to constant temperatures and fluctuating daily temperatures in an annual killifish *Austrofundulus limnaeus*. Journal of Experimental Biology, **207**, 2237-2254.

Ptashne M, Gann A (1997) Transcriptional activation by recruitment. Nature, **386**, 569-577.

Quinn TP (2005) The Behavior and Ecology of Pacific Salmon and Trout. University of Washington Press, Seattle, WA.

Raida MK, Buchmann K (2008) Development of adaptive immunity in rainbow trout, *Oncorhynchus mykiss* (Walbaum) surviving an infection with *Yersinia ruckeri*. Fish and Shellfish Immunology, **25**, 533-541.

Rifkin SA, Houle D, Kim J, White KP (2005) A mutation accumulation assay reveals a broad capacity for rapid evolution of gene expression. Nature, **438**, 220-223.

Schluter D (2001) Ecology and the origin of species. Trends in Ecology and Evolution, **16**, 372-380.

Schwanhausser B, Busse D, Li N, Dittmar G, Schuchhardt J, Wolf J, Chen W, Selbach M (2011) Global quantification of mammalian gene expression control. Nature, **473**, 337-342.

Sommer S (2005) The importance of immune gene variability (MHC) in evolutionary ecology and conservation. Frontiers in Zoology, **2**, 16.

St-Cyr J, Derome N, Bernatchez L (2008) The transcriptomics of life-history trade-offs in whitefish species pairs (*Coregonus* sp.). Molecular Ecology, **17**, 1850-1870.

Taylor EB (1991) A Review of Local Adaptation in Salmonidae, with Particular Reference to Pacific and Atlantic Salmon. Aquaculture, **98**, 185-207.

Tonteri A, Vasemägi A, Lumme J, Primmer CR (2010) Beyond MHC: signals of elevated selection pressure on Atlantic salmon (*Salmo salar*) immune-relevant loci. Molecular Ecology, **19**, 1273-1282.

Tuomi JM, Voorbraak F, Jones DL, Ruijter JM (2010) Bias in the Cq value observed with hydrolysis probe based quantitative PCR can be corrected with the estimated PCR efficiency value. Methods, **50**, 313-322.

Whitehead A, Crawford DL (2006) Neutral and adaptive variation in gene expression. Proceeding of the National Academy of Sciences USA, **103**, 5425-5430.

Wiseman S, Osachoff H, Basset E, Malhotra J, Bruno J, VanAggelen G, Mommsen TP, Vijayan MM (2007) Gene expression in the liver during recovery from an acute stressor in rainbow trout. Comparative Biochemistry and Physiology D, **2**, 234-244.

Young KA (2004) Toward evolutionary management lessons from salmonids. In Evolution Illuminated: Salmon and Their Relatives. Eds. Hendry AP, Stearns SC. Oxford University Press, New York. Pp. 358-376.

CHAPTER II

ENVIRONMENTAL ASSOCIATIONS WITH GENE TRANSCRIPTION IN BABINE LAKE RAINBOW TROUT: EVIDENCE FOR LOCAL ADAPTATION[*]

Introduction

Local adaptation is characterized by local genotype advantage where individuals experience higher fitness on average in their local environment than any other possible environment in which they could exist (Kaweki and Ebert 2004). Local adaptation implies that local environmental forces have acted, via natural selection, to increase traits that are advantageous to individuals in that environment. In salmonids, local adaptation is facilitated by high levels of natal philopatry and population sub-division that occurs across a landscape of variable environments (Quinn 2005). The scale and extent to which local adaptation occurs in salmonid populations appears to be context and trait dependent (Fraser et al. 2011) and is affected by the complex interactions of selection and drift within populations, and gene flow among populations. However, local adaptation is primarily thought to be a response to environmental variation, and indeed recent reviews have highlighted the roles temperature and diseases play in determining functional divergence among populations (Garcia de Leaniz et al. 2007; Fraser et al. 2011). A better understanding of the patterns and processes that affect the development and maintenance of local adaptation is critical to our understanding of the initiation of speciation processes (Schluter 2000) as well as the effective conservation of locally adapted populations (Fraser and Bernatchez 2001).

[*] Wellband KW, Heath DD Environmental associations with gene transcription in Babine Lake rainbow trout: Evidence for local adaptation. Submitted to Molecular Ecology.

Despite the use of quantitative genetics to study the genetic architecture of local adaptation (e.g. Aykanat et al. 2012) the molecular genetic mechanisms of local adaptation are currently not well characterized. Several indirect methods for exploring the genetic mechanisms contributing to local adaptation have been developed, including comparisons of population divergence at functional versus neutral loci (Q_{ST} vs F_{ST}) and correlations of environmental variables or gradients with phenotypic/genetic traits (reviewed by Fraser et al. 2011). However, more direct approaches have become feasible with the advent of rapid, cost-effective gene transcription assay methods. Measures of gene transcription have recently been shown to be powerful tools to investigate the molecular genetic nature of local adaptation because transcription: 1) is a heritable phenotype and 2) has direct consequences for an organism's growth, development and response to stimuli (Fay and Wittkopp 2008). Gene transcription profiles have been used to demonstrate local adaptation in a variety of ways. Parallel evolution of transcription profiles has been demonstrated among sympatric whitefish species pairs (Derome et al. 2006, St-Cyr et al. 2008). Breakdown of gene transcription among wild-farmed hybrids have been shown in Atlantic salmon (Normandeau et al. 2009, Tymchuk et al. 2010). Gene transcription profiles have also been linked to fitness of wild Sockeye salmon (Miller et al. 2011) and targeted studies of candidate loci transcription have also had success in detecting signatures of rapid evolution in natural populations (Jeukins et al. 2009, Aykanat et al. 2010).

The utility of gene transcription for identifying differences among populations is clear; however, few studies have been able to attribute divergence among populations to specific local environmental variation. Selective forces influencing phenotypic variation

in salmonid populations may include biotic and/or abiotic components of the environment (Taylor 1991, Garcia de Leaniz et al. 2007). Abiotic conditions associated with stream size (e.g. water temperature, flow, etc.) are important in explaining among-group phenotypic variation (reviewed by Garcia de Leaniz et al. 2007). Salmonid populations persist under a wide range of stream temperatures (e.g. Elliot et al. 1998) some of which are near critical thermal maxima for these species during summer. Coping with the metabolic demands to survive such temperature stresses is thus a potentially locally adaptable trait in situations where temperature regimes differ among populations. The primary response to metabolic stress in fish involves stimulation of the hypothalamus-pituitary-interrenal axis resulting in the release of glucocorticoids such as cortisol (Mommsen et al. 1999). Cortisol levels are heritable, can be differentially selected for and have consequences for fitness (Feldoven et al. 2002). In salmonids, cortisol release has been shown to trigger a reorganization of metabolism in the liver, mediated by gene transcription, that facilitates the rapid deployment of glucose to tissues providing the fuel to regain homeostasis (Wiseman et al. 2007).

The role of disease in driving salmonid local adaptation is also well established. Resistance and susceptibility to a variety of bacterial and parasitic infections has been associated with certain major histocompatibility (MH) alleles (e.g. Wynne et al. 2007: amoebic gill disease, Turner et al. 2007: bacterial kidney disease, Glover et al. 2007: sea lice, Dionne et al. 2009: myxozoa). Many of those studies were conducted under laboratory conditions in response to a single challenge. In contrast, MH heterozygosity has been associated with resistance to infection in salmon experiencing a complex bacterial community despite no single allele alone conferring resistance (Evans and Neff

2009) and evidence of selection at a variety of immune related loci has been demonstrated in natural populations (Tonteri et al. 2010) reinforcing the importance of studying immune system evolution under natural conditions. Few studies have characterized pathogen communities among natural salmon populations and relatively little is known about the spatial and temporal patterns of abundance of fish pathogens (McVicar et al. 2006); however, in general, microbial stream communities in temperate regions have stronger spatial than temporal structuring despite seasonal trends of succession (e.g. Hullar et al. 2006). The strength and direction of selection on the immune system varies across different life stages of salmon (de Eyto et al. 2011) indicating that if life stage-specific local adaptation to pathogens occurs, much of it would likely be in the first year of life, as juvenile salmonids experience high mortality during this period. Recognition of pathogens and the subsequent immune response is triggered through a complex set of receptors and signaling molecules (Medzhitov and Janeway 1997). A critical component of those pathways are small signaling proteins, cytokines and chemokines, which direct how the immune system responds to pathogens (Secombes et al. 1996; Bird et al. 2006). Transcriptional control of cytokine and chemokine activity has been documented in various tissues in fish (Raida and Buchmann 2006, 2008, Scapigliati et al. 2006) thus selection has the potential to act upon transcription of these signaling molecules.

The environmental factors expected to drive selection among habitats coupled with our understanding of gene function makes it possible to select candidate genes to test for specific functional divergence based on environmental variation among putatively locally adapted populations. Here we test the hypothesis that gene transcription at

candidate loci differs among genetically structured populations, and that attributes of the local environment are correlated with specific gene transcriptional profiles. Specifically, we investigate the role that temperature variation and bacterial community diversity play in determining gene transcription variation at biologically relevant loci among naturally occurring rainbow trout populations from Babine Lake, British Columbia. We use real-time quantitative polymerase chain reaction (RT-qPCR) to quantify gene transcription combined with next-generation pyrosequencing to quantify bacterial community diversity and one year of temperature data to provide evidence that local environments drive transcriptional difference, and ultimately, the evolution of local populations. This work provides insight into the mechanisms controlling local adaptation of salmon populations, with implications for how we view adaptation and the management of this species.

Methods

Sampling sites and protocol

We sampled six tributaries of Babine Lake (Figure 2.1) known to have rainbow trout spawning populations (Bustard 1989). In Babine Lake, rainbow trout spawn in late May and early June in over 34 tributaries, fry emerge from the gravel during mid-July to the first week of August and rear for up to three years in the stream before descending to the lake to spend their life as adults (Bustard 1989). Tributaries were chosen to represent a range of environmental conditions and watersheds, as well as geographic distances from one another (Bustard 1989, Koehler 2010). Tsak (TS) and 11 Mile (11M) creeks are small tributaries at the northern end of Babine Lake, Tachek (TA) and Cross (CR) creeks are medium and small tributaries respectively located near the midpoint of the lake, and

Duncan (DU) creek and the Sutherland River (SU) are small and large tributaries respectively, which drain a large watershed at the southern end of Babine Lake (Figure 2.1, Table 2.1). Stream temperature at the time of fish collection ranged from a high of 11.5 °C in Duncan creek to 8.5 °C in 11 Mile creek and followed a decreasing trend with increasing latitude (SU = 11.0 °C, CR = 10.0 °C, TA = N/A, TS = 9.0 °C). Genetic population structure has been demonstrated among all the tributaries we sampled (Koehler 2010) indicating reduced gene flow and the potential for the evolution of adaptive divergence.

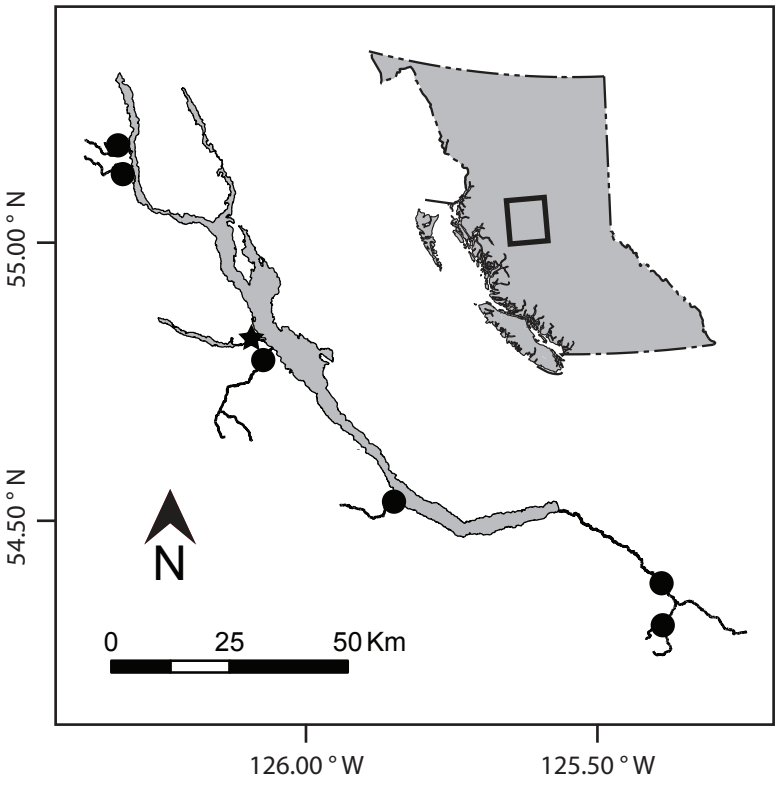

Figure 2.1: Map of Babine Lake and tributaries sampled (closed circles) for juvenile rainbow trout. The Fulton River facility where the fish were held and experiments were conducted is indicated by the solid star.

Table 2.1: Temperature profile and eubacterial community characterization of six Babine Lake tributaries ordered from north to south along the axis of the lake. Lat – latitude (decimal degrees), long – longitude (decimal degrees), FL – fork length range (mm) of sampled rainbow trout fry, MT – maximum temperature (°C), MDR – maximum daily temperature range (°C), ADR – average daily temperature range (°C), DA5 – first day with average temperature above 5 °C, Area – watershed area (Km^2), All 16S – total number of 16S rRNA sequences per library/tributary, 16S genus – number of 16S rRNA sequences identified to genus per library/tributary, Genera – number of genera detected.

Tributary	Lat (°N)	Long (°W)	FL (mm)	MT (°C)	MDR (°C)	ADR (°C)	DA5 (m/dd)	Area (Km^2)	All 16S	16S genus	Genera
Tsak	55.13884	126.61987	39 - 59	14.5	4.0	1.0	5/26	24	621	181	42
11 Mile	55.17806	126.62614	39 - 53	13.5	4.0	1.2	5/26	36	1028	324	51
Tachek	54.78710	126.12808	32 - 64	NA	NA	NA	NA	105	3925	1020	41
Cross	54.51376	125.70652	36 - 59	10.5	2.0	1.0	5/26	39	1942	476	70
Sutherland	54.33987	124.83503	37 - 58	11.5	2.0	0.8	5/21	1310	2358	562	70
Duncan	54.26835	124.84741	40 - 54	12.5	3.5	1.3	5/22	83	1395	479	27

Approximately 50 young-of-the-year (32 – 64 mm) rainbow trout (*Oncorhynchus mykiss* Walbaum) were collected from each tributary by dip-netting and electroshocking (Smith-Root BP-15 backpack shocker). Fish were placed into heavy plastic bags (60 x 120 cm) containing ambient water from their tributary. The bags were twisted closed to remove any ambient air and oxygen was then bubbled into the water and allowed to accumulate until it filled approximately ¼ the volume of water in the bag. Bags were sealed and transported (2 – 6 hours) on ice to the Department of Fisheries and Oceans' Fulton River Spawning Channel facility. Fish from each tributary were held in separate cages in a 3 m round tank with water flow-through from Fulton Lake (15 ± 0.5 °C). Fish were held for 5 days under starvation to acclimate to hatchery conditions and recover from the capture and transportation stress. Mortality only occurred for several individuals from one population (Tachek Creek). It is believed that those individuals were chronically stressed prior to sampling, as many dead fish were observed at the Tachek Creek sampling location.

Experimental protocol

Immune and temperature challenges were conducted on a subset of 10 fish from each population. The immune challenge consisted of fry being immersed in a 10% Vibrogen 2 vaccine bath containing formalin-inactivated cultures of *Vibrio anguillarum* serotypes I and II and *Vibrio ordalii* (Novartis Animal Health, Mississauga, Ontario) for one minute. The temperature challenge consisted of placing a different subset of fry in a water bath of 20 ± 0.5 °C water for one hour. The water temperature was chosen to be 5 °C above the ambient temperature of hatchery water but below the thermal maximum for

rainbow trout. Following both treatments, fry were returned to the holding tank. Control fish were handled in the same manner as challenged fish but did not experience the challenge to give an estimate of resting state transcription. Sampling of tissues occurred for control groups prior to exposure and for challenged groups 24 hours post-exposure. All fish were humanely euthanized using an overdose solution of clove oil (250 ppm) and gill tissues were dissected, immediately preserved in RNAlater and stored at 4 °C. Samples were frozen at -20 °C within 5 days and stored at that temperature until further analysis.

Selection of candidate loci

Wiseman et al. (2007) identified several differentially regulated genes in rainbow trout liver during metabolic stress (cathepsin D, glucocorticoid receptor (GR), pyruvate kinase (PK) and phosphoenolpyruvate carboxykinase (PEPCK)). We chose those genes as characteristic of major metabolic pathways that we assay for salmonid transcriptional response to a temperature stress. The function of PEPCK and PK were inferred from studies on all levels of life and are widely accepted to control rate-limiting steps of gluconeogenesis and glycolysis pathways (Pilkis and Granner 1992). Cathepsins are a class of proteolytic enzymes involved in protein degradation pathways where cathepsin D is the primary cathepsin responsible for intracellular protein degradation in lysosomes (Fusik and Vetvicka 2005). Glucocorticoid receptors are central to the activation of a stress response through cortisol signaling and have been widely studied in fish, including rainbow trout (Aluru and Vijayan 2009). Raida and Buchmann (2006, 2008) identified cytokine/chemokines as significantly up-regulated following an immune challenge in

rainbow trout (IL-1β, CXCL-8, IFNγ, TNFα), and those genes play important roles in determining downstream responses of the immune system. IL-1β and TNFα are involved in activating and modulating responses of the immune system by inducing inflammation and altering expression of other cytokines in fish (Whyte 2007). CXCL-8 is involved in the recruitment of immune effector cells to sites of infection (Whyte 2007) and IFNγ plays important roles in modulating growth, maturation and differentiation of various immune cells as well as activation of macrophages for killing bacterial and viral pathogens (Robertson 2006). We utilize these genes to assay immune response among populations.

RNA extraction and cDNA synthesis

Total RNA was extracted from gill tissue using mechanical homogenization of tissue in 0.8 mL of TRIZOL (Invitrogen) following the method of Chomczynski and Sacchi (1987). Total RNA preparations were assessed for quality using gel-electrophoresis where clear 28S and 18S rRNA bands and minimal low-molecular-weight smear indicated good quality RNA. Purity and concentration of total RNA was assessed using UV spectrophotometry in a Victor 3V plate reader (Perkin Elmer). All total RNA preparations had purity values of 1.9-2.1 (A260/A280). Based on the concentration calculated using UV spectrophotometry, total RNA was diluted to 100ng/uL and treated with DNase 1 (Fermentas) to remove genomic DNA contamination. Total RNA was converted to complementary DNA (cDNA) using a High Capacity cDNA kit (Applied Biosystems). Reverse transcriptase (RT) reactions contained 1.0 ug of total RNA, 2 uL of random primers (Applied Biosystems), 4 mM each dNTP, 50 U of MultiScribe reverse

transcriptase (Applied Biosystems) and 40 U of RNase Inhibitor (Applied Biosystems) in a 1X RT buffer at a final volume of 20 uL. RT reactions were incubated at 25 °C for 10 minutes followed by 37 °C for 2 hours and were stopped by incubating at 85 °C for 5 minutes. RNA from DNA-RNA hybrids was degraded by using 1 U of RNAase H (New England Biolabs) for each RT reaction and incubation at 37 °C for 20 minutes. RT reactions were then diluted to a final volume of 100 uL with ddH_2O.

Quantitative real-time PCR

Four biologically relevant genes for each treatment and two reference genes (Table 2.2) were assayed in six individuals from each population for each treatment. Primers and probes for previously unpublished loci were designed using publicly available cDNA sequences from GenBank and Primer Express software (Applied Biosystems). Where possible, primers were designed across exon-intron boundaries to reduce amplification of residual genomic DNA contamination. Both reference genes have been shown to be stably expressed before and after stress challenges (Ortega et al. 2005, Ching et al. 2010). PCR reactions contained 50 nM Taqman probe, 100 nM forward and reverse primers and 10 ng of cDNA in a 1X master mix (Taqman Gene Expression master mix, Applied Biosystems). Assays were run in triplicate for reference genes and in duplicate for target genes on an ABI 7500 Real-Time PCR machine (Applied Biosystems) for 45 cycles of 95°C for 30 s and 60°C for 1 min.

Table 2.2: Primers and probes for quantitative real-time PCR assays of rainbow trout candidate gene transcription.

Gene	Treatment	Accession	Forward Seq	Reverse Seq	MGB probe Seq	Reference
EF-1α	Reference	AF498320	AATACCCTCCTCTTGGTCGTTTC	CTTGTCGACGGCCTTGATG	TGCGTGACATGAGGC	Aykanat et al. 2011
ARP	Reference	AY685220	TTGTTTGACTAACTTGCTATTCTTTGC	CGCCGACAATGAAACATTTG	AATTGCTGGATGACTATC	Ortega et al. 2005
CathepsinD	Temperature	U90321	GGGAGGAACTGACCCGAAGT	GCGGCTGACGTCGAGGTA	CTACAGTGGAGACTTCCA	This study
GR	Temperature	Z54210	CTGGCTGTTCCTCATGTCGTT	CAACATCCCCCCGTTACACT	CTTGGGCTGGCGCT	This study
PEPCK	Temperature	AF246149	GCCCCTTCTTCGGCTACAA	CTTGCGGGTCTCCATGCT	TCGGTGACTACCTAGCC	This study
PK	Temperature	AF246146	TGGGCCGACGATGTAGACA	CCCCTGGCCTTTCCTATGTT	CAGAGTCAACTTCGGC	This study
IL-1β	Immune	AJ223954; AJ298294	ACATTGCCAACCTCATCATCG	TTGAGCAGGTCCTTGTCCTTG	ATGGAGAGGTTAAAGGGT	Raida & Buchmann 2008
CXCL-8	Immune	AJ279069	AGAATGTCAGCCAGCCTTGT	TCTCAGACTCATCCCCTCAGT	TTGTGCTCCTGGCCCT	Raida & Buchmann 2008
IFNγ	Immune	AY795563	CAAACTGGCCCTTAAGTTCCA	TCTGGGCTTGCCGTCTCT	TAAAGAAGGACAACCGC AGG	Aykanat unpublished
TNFα	Immune	AJ277604; AJ401377	GGGGACAAACTGTGGACTGA	GAAGTTCTTGCCCTGCTCTG	ACCAATCGACTGACCGAC	Raida & Buchmann 2008

PCR efficiency for each amplicon was determined using the program LinRegPCR (Ramakers et al. 2003) and amplicon efficiency, threshold and Cq values were obtained and used to calculate theoretical starting cDNA concentrations (N_0) per technical replicate in LinRegPCR (Ramakers et al. 2003) using the unbiased method of Tuomi et al. (2010) for hydrolysis (TaqMan) probes. Technical replicates for genes were averaged within individuals. Reference genes (EF-1α and ARP) were combined to create a normalization factor by taking the geometric mean of the N_0 values for the reference genes within individuals. Transcription of target genes was then expressed as a ratio of the value for the gene relative to the normalization factor.

Tributary environment characterization

Microbial community: One liter of water was collected from each sampled tributary and filtered through 0.2 um filters (Pall Life Sciences). Environmental DNA (eDNA) was extracted from each sample using a modified phenol:chloroform and a CTAB buffer extraction (Chaganti et al. 2012) with 3 freeze-thaw cycles and mechanical homogenization to lyse bacterial cells. A 278 base pair portion of the 16 S ribosomal gene that contains the V6 variable region (for taxonomic identification) was amplified with primers corresponding to 786-1063 bp of the *E. coli* 16 S gene (Forward primer sequence: GATTAGATACCCTGGTAG, Reverse primer sequence: CTCACGRCACGAGCTGACG). Polymerase Chain Reactions (PCRs) were performed in a 25 uL volume and contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM $MgCl_2$, 200 uM each dNTP, 0.4 uM primers, 1 U of AmpliTaq DNA polymerase (Applied Biosystems) and 50 – 100 ng of eDNA. Reactions were amplified for 25 cycles of 94°C

for 30 s, 56°C for 30 s and 72°C for 40 s. The PCRs were then split and amplified in triplicate for 20 cycles using adaptor-modified (for 454 pyrosequencing sequencing) primers following the same conditions. PCR products were gel-purified, standardized with respect to concentration and pooled. Emulsion PCR was completed by Engencore (Columbia, South Carolina) and sequencing was performed on a GS FLX pyrosequencer (454 Life Sciences). Raw pyrosequencing data was processed, primer sequences trimmed and low-quality sequences removed using the RDP pyrosequencing pipeline (Cole et al. 2008). Processed sequences were then classified using the online RDP naive Bayesian rRNA classifier (Wang et al. 2007) with a conservative confidence threshold of 80%.

Temperature profile: Temperature data loggers (iBCod DS1922L, Maxim Integrated Products) were deployed in each tributary during initial sampling (August 2010) and recovered from 5 of the 6 tributaries the following spring (May 2011; one data logger was lost over the winter). Data loggers recorded water temperature every 4 hours to an accuracy of 0.5 °C. Maximum and minimum water temperatures, average and maximum daily range of water temperature and the first day with average water temperature above 5°C were calculated.

Data analysis

All analyses were conducted in the statistical software R version 2.14.1 (R Development Core Team 2011). First, we tested for a correlation between gene transcription values and fork length for each gene assayed to assess the role body size played in gene transcription. We also correlated gene transcription values with geographic order (South to North) to assess potential geographic influences on gene

transcription because isolation by distance has been demonstrated for these populations using neutral microsatellite markers (Koehler 2010). To test for transcriptional response to our challenges, we used T-tests to test for differences between control and challenged transcription for each gene in each population. To account for multiple tests we calculated the false discovery rate for each challenge and global p-values for each locus using 1000 random permutations of the data. P-values were calculated by dividing the number of permutations with a p-value less than the observed p-value for the original data by the total number of permutations. False discovery rates were calculated for each challenge as the random expectation of the number of significant tests per permutation divided by the number of observed significant tests in the original data. To test for population differences in response to stress we subtracted mean population resting state transcription from challenged individuals. We then compared population responses in a one-way ANOVA for each gene.

We conducted principle components analysis on transcription data from metabolic and immune genes separately for non-challenged and response to challenge groups. Response to challenge was calculated by subtracting the mean transcription of the control group for each population from each challenged individual in that population. We also conducted separate principle components analysis on the water temperature data and relative abundance of bacterial genera for each stream. Due to missing stream temperature data, the Tachek creek samples were omitted from the metabolic gene and stream temperature analyses. We investigated correlations among the major axes of variation (principal components) in gene transcription and the environmental datasets using co-inertial analyses (Doledec and Chessel 1994, Culhane et al. 2003) in the ade4

package in R (Dray and Dufour 2007). Co-inertia analysis tests for correlations among the axis of separate ordinations (e.g. principal components analysis) for the same samples (in this case gene transcription and environmental variables). The significance of association resulting from the co-inertial analyses was assessed using 1000 random permutations of the data.

Results

Gene transcription

Response to challenge: All metabolic and immune genes were up-regulated following challenge in at least one population. Cathepsin D and glucocorticoid receptor were up-regulated in multiple populations while PEPCK and pyruvate kinase only increased significantly in one population (Tachek and Sutherland respectively; Figure 2.2). Of the immune genes assayed IL-1β, IFNγ and TNFα transcription was significantly increased after challenge in multiple populations and CXCL-8 only increased in one population (Tachek; Figure 2.2). The only gene that had a significant global response was IL-1β ($p = 0.041$). False discovery rates were calculated to be very low (temperature: FDR = 0.024, immune: FDR = 0.015) indicating that, despite multiple tests, the significance of our results are not obscured by false positives. These results indicate that the challenges we chose do induce transcriptional responses but that the response is population-specific (Figure 2.2, Table 2.3). To support this argument, results from one-way ANOVAs indicate that population level transcriptional response to challenge differed significantly for all genes assayed (Cathepsin D: $p = 0.032$, GR, PEPCK, PK, IL-1β, CXCL-8, IFNγ and TNFα all: $p < 0.001$).

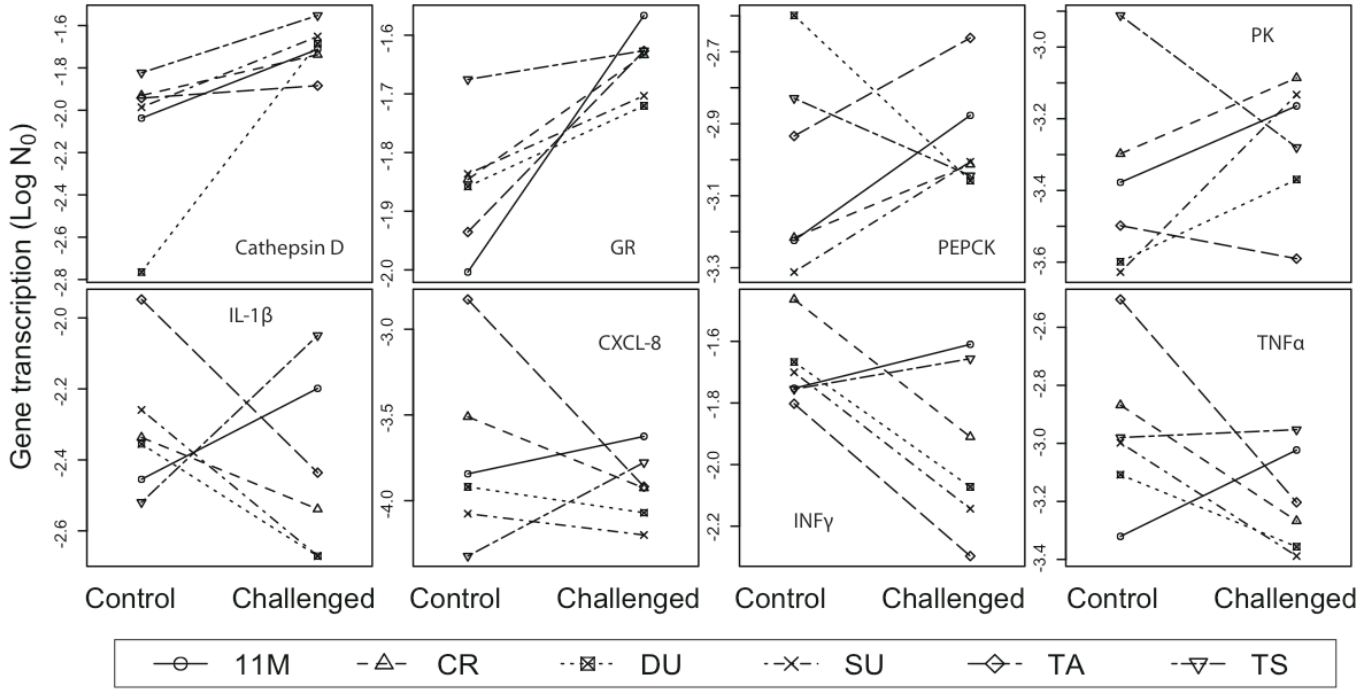

Figure 2.2: Gene transcription reaction norms for candidate loci from six Babine Lake tributary populations in response to temperature stress (top row) and immune challenge (bottom row).

Table 2.3: Mean gene transcription values for candidate loci (Log_{10} Mean (SE)) among populations at resting state (Control) and induced (Treatment) transcription levels.

Population	cathepsin D	GR	PEPCK	PK	IL-1β	CXCL-8	IFNγ	TNFα
Control								
11 Mile	-2.04 (0.09)	-2.00 (0.05)	-3.22 (0.16)	-3.38 (0.23)	-2.45 (0.12)	-3.84 (0.13)	-1.75 (0.10)	-3.32 (0.08)
Cross	-1.93 (0.08)	-1.85 (0.08)	-3.22 (0.07)	-3.30 (0.10)	-2.34 (0.11)	-3.51 (0.22)	-1.46 (0.13)	-2.87 (0.11)
Duncan	-2.76 (0.26)	-1.86 (0.02)	-2.60 (0.30)	-3.60 (0.11)	-2.36 (0.04)	-3.92 (0.06)	-1.67 (0.03)	-3.11 (0.07)
Sutherland	-1.99 (0.07)	-1.84 (0.07)	-3.31 (0.16)	-3.63 (0.09)	-2.26 (0.14)	-4.08 (0.11)	-1.70 (0.21)	-3.00 (0.23)
Tachek	-1.94 (0.14)	-1.94 (0.06)	-2.93 (0.08)	-3.50 (0.14)	-1.95 (0.16)	-2.83 (0.35)	-1.80 (0.10)	-2.50 (0.25)
Tsak	-1.82 (0.11)	-1.68 (0.10)	-2.83 (0.16)	-2.91 (0.22)	-2.52 (0.06)	-4.32 (0.23)	-1.76 (0.16)	-2.98 (0.12)
Treatment								
11 Mile	-1.71 (0.05)	-1.57 (0.02)	-2.88 (0.03)	-3.16 (0.04)	-2.20 (0.07)	-3.62 (0.06)	-1.61 (0.07)	-3.02 (0.05)
Cross	-1.74 (0.04)	-1.63 (0.05)	-3.01 (0.11)	-3.09 (0.15)	-2.54 (0.05)	-3.93 (0.07)	-1.91 (0.08)	-3.27 (0.10)
Duncan	-1.68 (0.03)	-1.72 (0.05)	-3.06 (0.07)	-3.37 (0.08)	-2.67 (0.06)	-4.07 (0.06)	-2.07 (0.08)	-3.36 (0.10)
Sutherland	-1.65 (0.06)	-1.70 (0.04)	-3.01 (0.11)	-3.13 (0.20)	-2.67 (0.05)	-4.20 (0.09)	-2.14 (0.06)	-3.39 (0.05)
Tachek	-1.88 (0.15)	-1.63 (0.05)	-2.66 (0.08)	-3.59 (0.26)	-2.44 (0.12)	-3.92 (0.19)	-2.30 (0.16)	-3.20 (0.10)
Tsak	-1.55 (0.02)	-1.63 (0.05)	-3.04 (0.04)	-3.28 (0.08)	-2.05 (0.04)	-3.78 (0.10)	-1.66 (0.05)	-2.95 (0.06)

Gene transcription does not correlate with body size (fork length) nor with the order in which sites occur along the axis of the lake for any of the genes or treatments we investigated, suggesting that systematic sampling biases do not influence transcriptional variation. Isolation by distance has been previously demonstrated for the populations in this study using neutral microsatellite markers (Koehler 2010) and the lack of geographic patterns in gene transcription suggests that genetic drift is not driving transcriptional variation among populations.

Tributary environment characterization

Eubacterial 16S rRNA libraries were obtained for all six streams sampled in 2011. Sizes of the trimmed and quality filtered sequence libraries ranged from 503 – 3419 sequences (Table 2.1). Of those sequences the RDP classifier assigned between 181 – 1020 (27-41%) sequences per library to the taxonomic level of genus, the lowest level of classification obtainable. The number of genera detected ranged from 27 – 70 among tributaries (Table 2.1). We used only the sequences assigned to a genus for the following analyses. For each sequence library 27 – 620 (8-61%) sequences were assigned to genera that contain at least one species suspected to cause disease in fish. In total across all 16S rRNA sequence libraries, 1174 sequences representing potentially pathogenic organisms were detected. *Flavobaterium* accounted for 61% (774 sequences) of all potentially pathogenic organisms sequences detected followed by *Acidovorax* (13%, 150 sequences) and *Corynebacterium* and *Streptococcus* (5%, 57 sequences each). The remaining 16% of sequences were accounted for by 11 genera and their abundances ranged from 1 – 32

individuals detected across all libraries. The proportion of Flavobacterium sequences in each library ranged from 0 – 50% (0 – 510 sequences).

Temperature loggers were deployed and successfully recovered from 5 of the 6 tributaries. Data loggers were deployed for 272 – 285 days spanning a period from late August of 2010 until late May of 2011. Data loggers were deployed in the deepest pools to prevent them from freezing; however, on or about November 8, 2010 all recovered loggers reached low temperatures at 0.0 °C and the temperature did not change until the following April. As a result the average daily range we present only represents the period of time during which water temperatures were recorded to be above 0°C. Maximum recorded temperature, maximum daily range and the average daily range varied among tributaries (Table 2.1). The first day with a mean daily water temperature above 5 °C also varied by as many as 5 days among tributaries, which may be associated with rainbow trout spawning run timing (Bustard 1990) and egg/fry development.

Transcription – environment associations

Principal components analysis of metabolic gene transcription in control and response to challenge groups identified the first two axis of variation that respectively explained 76% and 73% of the variation in the data (Control: PC1 = 47%, PC2 = 29% and Challenged: PC1 = 40%, PC2 = 33%). For the control group, PC1 was loaded primarily by cathepsin D and glucocorticoid receptor and PC2 was loaded primarily by PEPCK. In the challenged group, PCA loadings indicated PC1 was loaded equally by glucocorticoid receptor, PEPCK and PK and PC2 was loaded primarily by cathepsin D. Principal components analysis of the immune gene transcription in control and response

to challenged groups each explained 93% or 96% of the variation respectively and both identified two major axes of variation in the data (Control: PC1 = 68%, PC2 = 25% and Challenged: PC1 = 69%, PC2 = 27%). Loadings for the immune genes were much more ambiguous, and three genes contributed approximately equally to PC1 for both experimental groups (IL-1β, CXCL-8, TNFα) and one gene each loaded onto PC2 (IFNγ).

The first two principal components of the stream temperature dataset (maximum stream temperature, maximum daily range, average daily range and first day with average temperature obove 5 C) explained 76% of the variation (PC1 = 47%, PC2 = 29%). The first axis was loaded primarily by maximum temperature, maximum daily range, and to a lesser extent average daily range. The second axis was loaded primarily by the first day with average temperature above 5 °C. The bacterial community analysis produced one axis that explained 86% of the variation in bacteria communities among sites. We retained the second axis (9% of variation) for ease of visualization.

The co-inertia analysis revealed a significant association between stream temperatures and metabolic gene transcription in the control treatment ($R^2 = 0.19$, $p = 0.013$). The strongest associations were PEPCK transcription with average daily temperature range and PK transcription with maximum stream temperatures (Figure 2.3). Cathepsin D and GR showed less strong negative associations with average daily temperature range. The co-inertia analysis of stream temperatures with response to temperature challenge gene transcription demonstrated even stronger associations ($R^2 = 0.39$, $p = 0.001$).

Co-inertia analysis of bacterial community and immune gene transcription again produced significant associations among both the control groups ($R^2 = 0.25$, $p = 0.009$) and challenged fish ($R^2 = 0.65$, $p = 0.001$). The strongest associations were between *Flavobacterium* and the genes IL-1β, CXCL-8, and TNFα (Figure 2.4). IFNγ exhibited a comparatively weak negative association with this genus. There were also strong associations between *Variovorax* and *Acidovorax* and the genes IL-1β, CXCL-8, and TNFα. IFNγ was positively associated with *Polynucleobacter* where IL-1β, CXCL-8, and TNFα were all negatively associated with this genus.

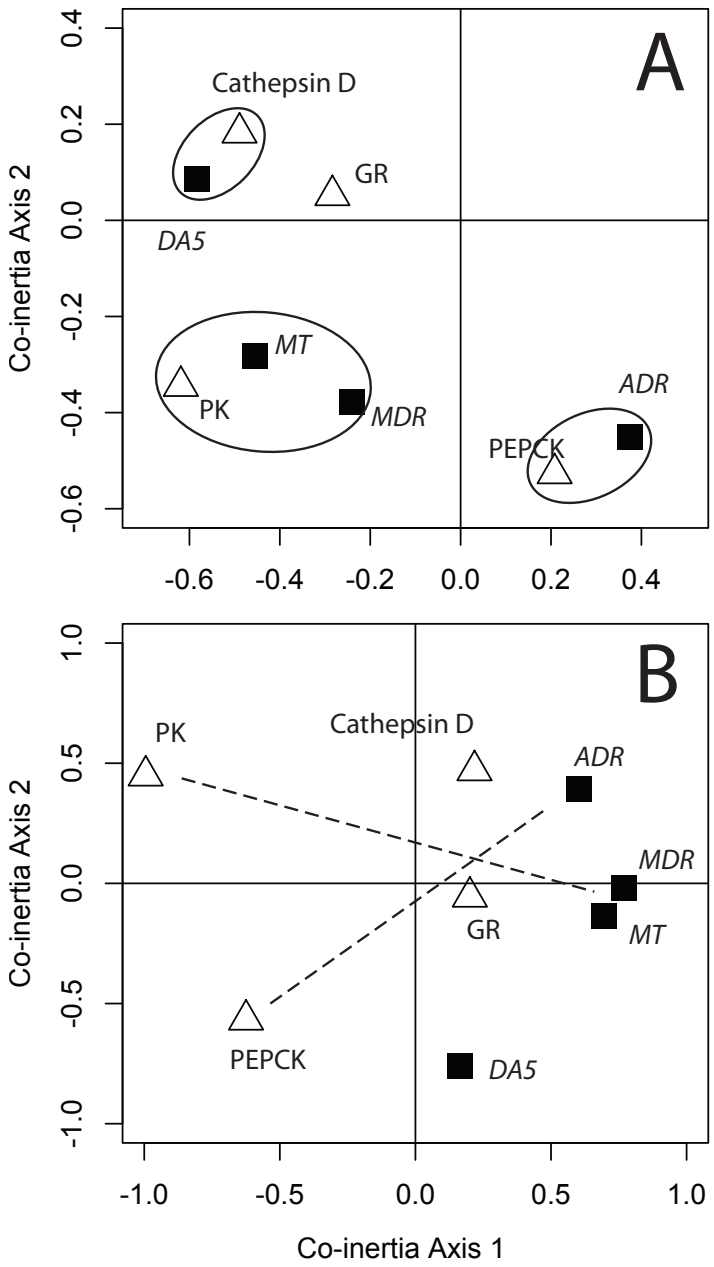

Figure 2.3: Co-inertia analysis of stream temperature profile (black squares, italic font) and resting state (A) or response to challenge (B) metabolic gene transcription (open triangles, regular font). Positive associations indicated by ellipses and negative associations indicated by dashed lines. See Table 2.1 for stream variable abbreviations.

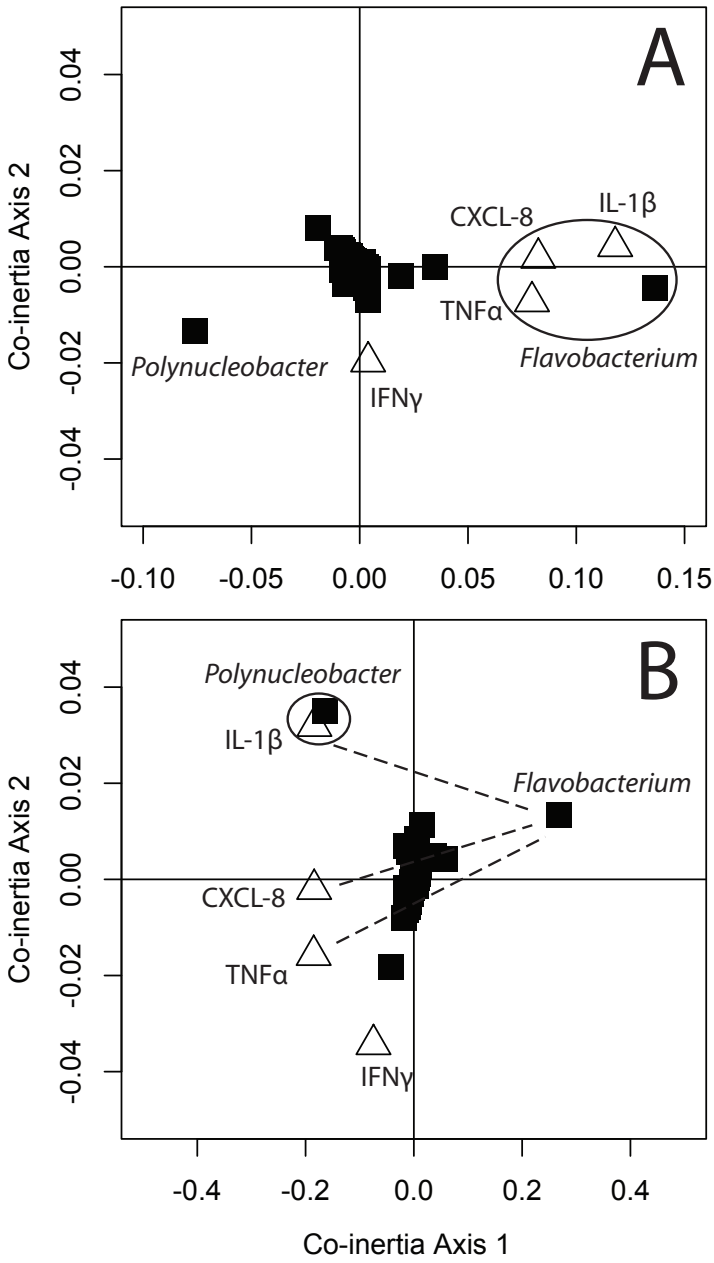

Figure 2.4: Co-inertia analysis of relative abundance of stream bacteria (black squares, italic font) and resting state (A) or response to challenge (B) cytokine/chemokine gene transcription (open triangles, regular font). Positive associations indicated by ellipses and negative associations indicated by dashed lines.

Discussion

The change in gene transcription at all loci in response to the challenges in our study indicates that the genes we studied are responding to stress. The different patterns of response we observed among populations suggest that diverging evolutionary processes (i.e., not stabilizing selection) are contributing to the variation among Babine Lake rainbow trout tributary populations. Population structure is present among Babine Lake rainbow trout tributary populations and neutral divergence in this system follows a pattern of isolation by distance (Koehler 2010); however, the lack of consistent clinal variation in gene transcription observed across a geographic gradient suggests, but does not preclude, that differences are not a result of genetic drift. Local adaptation can occur when gene flow is reduced among populations and the environmental conditions they experience differ (Kaweki and Ebert 2004). The tributary populations we studied indeed experience variation in temperature fluctuations and extremes as well as variation in the composition of microbial communities, suggesting that differential selection on gene transcription may explain the observed differences among populations. Transcriptional differences among natural salmonid populations have been demonstrated in the context of detrimental hybridization effects among locally adapted populations and aquaculture escapes (Normandeau et al. 2009; Tymchuk et al. 2011) as well as life-history trade-offs among species pairs (St-Cyr et al. 2008). Our results are consistent with the conclusion that gene transcription profiles are population-specific, and hence may reflect local adaptation in the early rearing habitat of Babine Lake rainbow trout; however, phenotypic differences observed among isolated populations do not constitute strong evidence for

local adaptation. To make a stronger case for local adaptation we show that gene transcription is correlated with stream environments.

The range of temperatures recorded for our streams are consistent with those measured for other salmon-bearing streams (Elliot et al. 1998). Temperature ranges comparable to those we recorded have been investigated as drivers of selection on growth rates (Jonsson et al. 2001). Jonsson et al. (2001) failed to demonstrate a correlation between optimal growth temperature of juvenile Atlantic salmon and the thermal conditions of their streams; however, they suggested that differences in growth efficiency among populations might be linked, in part, to thermal conditions of the streams. Trade-offs between transcription of growth or survival (stress response) genes have been demonstrated among whitefish species pairs adapted to benthic and limnetic habitats (St-Cyr et al. 2008). Among other species of fish, gene transcription mediated adaptation to different temperature regimes has been demonstrated for metabolic genes, including pyruvate kinase, in *Fundulus heteroclitus* (Whitehead and Crawford 2006). Furthermore, experiments with wild caught *Fundulus* have also demonstrated greater differences among populations at resting state than after a heat shock (Healy et al. 2010) consistent with the findings of our study. Perhaps the optimal strategy for coping with a stressful event is strongly selected upon and populations evolve to maintain different resting state transcription to balance energetic costs against the frequency of stressful events. To this end, trade-offs between transcription of growth and stress response genes have been demonstrated for both chronic and fluctuating heat stress in *Fundulus* (Podrabsky and Somero 2004) reinforcing the role trade-offs may play in the local adaptation of gene transcription.

Positive associations of maximum stream temperature with resting state transcription of genes controlling glycolysis (PK) are consistent with results from *Fundulus* (Whitehead and Crawford 2006) and suggest a role for increased metabolism of glucose in coping with thermal extremes. This interpretation is also consistent with the results of Wiseman et al. (2007) who demonstrated gene transcription patterns in the livers of rainbow trout represent a reorganization of metabolism to facilitate the breakdown of energy-rich molecules and increased production of glucose for export to body tissues to cope with metabolic stress. The association of average daily temperature range with transcription of the rate-limiting enzyme for gluconeogenesis (PEPCK) indicates that experiencing larger fluctuations in temperature may require tissues to have a greater capacity to produce their own glucose. Until now, a direct link between stream temperature and local adaptation in salmonids had not been established (Garcia de Leaniz et al. 2007); however, it appears temperature can play a role in modulating selection for growth and survival traits. Studies from *Fundulus* have demonstrated additional transcriptional differences among populations related to temperature regimes of heat shock proteins (Fangue et al. 2006) and xenobiotic processing (Whitehead and Crawford 2006) which may be worthwhile investigating in future salmonid studies.

Despite our modest sample size for bacterial community analysis, we discovered a high level of diversity of microbial taxa in this system, consistent with other studies utilizing massively parallel 16S rRNA sequencing (e.g. Bolhuis and Stal 2011). Populations of fish from different streams are known to experience different microbial communities as these are often tied to bedrock geochemistry, water chemistry, temperature and surrounding terrestrial ecosystems (e.g. Hullar et al. 2006). Futhermore,

there is spatial diversity in the bacterial pathogens infecting juvenile salmonids (Dionne et al. 2009, Evans and Neff 2009). In the genera detected in our study, we identified several as potentially pathogenic: *Flavobacterium psychrophilum* is the cause of cold-water disease/rainbow trout fry syndrome (Lorenzen et al. 1997), several members of *Pseudomonas* are opportunistic pathogens known to cause lesions and death in juvenile salmonids (e.g. Altinok et al. 2006) and *Mycobacterium* species have been implicated as the cause of fish tuberculosis (Arakawa and Fryer 1984). Many disease-causing bacteria in fish are opportunistic pathogens that become virulent during periods of stress (Harvell et al. 2002), suggesting that infection by other previously undescribed pathogens are also possible. As a result, the strength of selection resulting from pathogen pressure on juvenile salmonids is inextricably linked to stream temperature because of the positive relationships between pathogen diversity, abundance and temperature (Harvell et al. 2002, Dionne et al. 2009). However, it is likely that pathogen-mediated selection on juvenile salmonids would exceed that of temperature alone due to high mortality rates associated with disease outbreaks in young-of-the-year salmon (Holt et al. 1989). The positive association of multiple cytokine gene transcription and *Flavobacterium* relative abundance we demonstrated suggests a role for natural selection in determining population level differences in transcription. The stronger correlation among immune gene transcription ($R^2 = 0.25$, $p = 0.009$) versus metabolic gene transcription ($R^2 = 0.19$, $p = 0.013$) and environments also supports possible stronger selection imposed by pathogens on juvenile salmonids. Multiple lines of evidence for selection by specific pathogens as well as pathogen diversity on MH and other immune related loci have been demonstrated for a variety of salmonid species (e.g. Dionne et al. 2007, 2009, Evans and

Neff 2009, Tontori et al. 2010, de Eyto et al. 2011). To our knowledge our work represents the first evidence of local adaptation mediated by transcription of immune system candidate genes in natural populations.

We found *Flavobacterium spp.* to be positively associated with IL-1β, CXCL-8, and TNFα gene transcription among populations of rainbow trout in Babine Lake, indicating that *Flavobacterium spp.* may be a potent selective agent in this system. One representative of this genus, *Flavobacterium psychrophilum*, is a cold-water pathogen that becomes virulent at low temperatures (Holt et al. 1989). It causes lesions and can result in up to 90% mortality for rainbow trout fry. The positive association between transcription of cytokines and relative abundance of *Flavobacterium spp.* indicates that populations may be trading off the energetic costs of transcribing cytokines with the frequency of infections they experience. Higher levels of resting state transcription in certain populations may reflect the fish's ability or need to respond transcriptionally to infection. A reduced transcriptional response of cytokine genes to a secondary infection has been demonstrated for juvenile rainbow trout that survived a primary infection (Raida and Buchman 2009). The reduced response Raida and Buchmann (2009) demonstrated was suggested to represent the development of adaptive immunity and a reduced reliance on innate immune cytokine transcriptional response. This would suggest that increased relative abundance of pathogens in the streams we studied may result in a negative association with cytokine transcription due to the presence of acquired adaptive immunity. The absence of this pattern in our transcription data could be explained by the incomplete immunity of juvenile rainbow trout (Johnson et al. 1982) or by the diversity of other opportunistic pathogens experienced by juvenile rainbow trout in a complex

natural environment. In addition, selection acting to drive differences in the timing of cytokine transcriptional response cycle may explain the down regulation observed in certain populations while other populations are observed to be up regulating transcription of these genes.

In contrast to *Flavobacterium psychrophilum*, increases in the diversity and virulence of opportunistic pathogens are generally correlated with increasing temperature (Harvell et al. 2002). Despite this, little else is known about the specific pathogenicity and conditions favoring opportunistic infection by many other bacteria (McVicar et al. 2006). As more immunological studies are conducted under both laboratory and natural conditions, we will have a clearer picture of the potential threats previously undescribed fish pathogens may pose for wild populations, as well as the dynamics of immune response in response to variable and complex natural environments (Pederson and Babayan 2011). A clear concern, as climates continue to warm, is the risk for more opportunistic infections to occur and create multiple stresses for fish species and populations already in decline (Crozier et al. 2008).

In conclusion, we provide evidence for the important role of gene transcription in mediating the process of local adaptation in tributary populations of rainbow trout. By providing a link between local environmental conditions and specific gene transcription profiles we have strengthened the case that rapid evolution to local environments occurs, and have provided insight into the mechanisms that facilitate local adaptation of natural populations. Specifically, we highlight the role of temperature as a selective force on the transcriptome of salmonids both directly, by affecting the thermal regime fish experience, and indirectly, by influencing co-existing pathogen communities. We also provide the

first evidence of local adaptation selection by pathogens on the transcription of immune related genes. In light of climate change, the strength of selection by these direct and indirect means will undoubtedly change in unpredictable ways, likely leading to complex response patterns to local environmental variation. Finally, the population-specific response to stress we report reinforces the functional variability among genetically structured populations and emphasizes the need to conserve individual tributary populations to maintain maximal levels of genetic diversity and hence evolutionary potential.

References

Altinok I, Kayis S, Capkin E (2006) *Pseudomonas putida* infection in rainbow trout. Aquaculture, **261**, 850-855.

Aluru N, Vijayan MM (2009) Stress transcriptomics in fish: A role for genomic cortisol signaling. General and Comparative Endocrinology, **164**, 142-150.

Arakawa CK, Fryer JL (1984) Isolation and characterization of a new subspecies of *Mycobacterium chelonei* infectious for salmonid fish. Helgolander Meeresuntersuchungen, **37**, 329-342.

Aykanat T, Bryden CA, Heath DD (2012) Sex-biased genetic component distribution among populations: additive genetic and maternal contributions to phenotypic differences among populations of Chinook salmon. Journal of Evolutionary Biology, **25**, 682-690.

Aykanat T, Thrower FP, Heath DD (2011) Rapid evolution of osmoregulatory function by modification of gene transcription in steelhead trout. Genetica, **139**, 233-242.

Bird S, Zou J, Secombes CJ (2006) Advances in fish cytokine biology give clues to the evolution of a complex network. Current Pharmaceutical Design, **12**, 3051-3069.

Bolhuis H, Stal LJ (2011) Analysis of bacterial and archaeal diversity in coastal microbial mats using massive parallel 16S rRNA gene tag sequencing. International Society of Microbial Ecology Journal, **5**, 1701-1712.

Bustard D (1989) Assessment of rainbow trout recruitment from streams tributary to Babine Lake. Consultant report prepared for Skeena Region Fisheries Branch, BC Ministry of Environment and Parks. Smithers, BC. 1-71.

Bustard D (1990) Sutherland River rainbow trout radio telemetry studies 1989. Consultant report prepared for Skeena Region Fisheries Branch, BC Ministry of Environment. Smithers, BC. 1-56.

Chaganti SR, Lalman JA, Heath DD (2012) 16S rRNA gene based analysis of the microbial diversity and hydrogen production in three mixed anaerobic cultures. International Journal of Hydrogen Energy, **37**, 9002-9017.

Ching B, Jamieson S, Heath JW, Heath DD, Hubberstey A (2010) Transcriptional differences between triploid and diploid Chinook salmon (*Oncorhynchus tshawytscha*) during live *Vibrio anguillarum* challenge. Heredity, **104**, 224-234.

Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Analytical Biochemistry, **162**, 156-159.

Cole JR, Wang Q, Cardenas E, Fish J, Chai B, Farris RJ, Kulam-Syed-Mohideen AS, McGarrell DM, Marsh T, Garrity GM, Tiedje JM (2008) The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. Nucleic Acids Research, **37**, D141-D145.

Crozier LG, Hendry AP, Lawson PW, Quinn TP, Mantua NJ, Battin J, Shaw RG, Huey RB (2008) Potential responses to climate change in organisms with complex life histories: evolution and plasticity in Pacific salmon. Evolutionary Applications, **1**, 252-270.

Culhane AC, Perrière G, Higgins DG (2003) Cross-platform comparison and visualization of gene expression data using co-inertia analysis. BMC Bioinformatics, **4**, 59.

de Eyto E, McGinnity P, Huisman J, Coughlan J, Consuegra S, Farrell K, O'Toole C, Tufto J, Megens HJ, Jordan W, Cross T, Stet RJM (2011) Varying disease-mediated selection at different life-history stages of Atlantic salmon in fresh water. Evolutionary Applications, **4**, 749-762.

Derome N, Duchesne P, Bernatchez L (2006) Parallelism in gene transcription among sympatric lake whitefish (*Coregonus clupeaformis* Mitchill) ecotypes. Molecular Ecology, **15**, 1239-1249.

Dionne M, Miller KM, Dodson JJ, Bernatchez L (2009) MHC standing genetic variation and pathogen resistance in wild Atlantic salmon. Philosophical Transactions of the Royal Society B, **364**, 1555-1565.

Dionne M, Miller KM, Dodson JJ, Caron F, Bernatchez L (2007) Clinal variation in MHC diversity with temperature: evidence for the role of host–pathogen interaction on local adaptation in Atlantic salmon. Evolution, **61**, 2154-2164.

Dolédec S, Chessel D (1994) Co-inertia analysis: an alternative method for studying species-environment relationships. Freshwater Biology, **31**, 277-294.

Dray S, Dufour AB (2007) The ade4 package: implementing the duality diagram for ecologists. Journal of Statistical Software, **22**, 1-20.

Elliot SR, Coe TA, Helfield JM, Naiman RJ (1998) Spatial variation in environmental characteristics of Atlantic salmon (*Salmo salar*) rivers. Canadian Journal of Fisheries and Aquatic Science, **55**, 267-280.

Evans ML, Neff BD (2009) Major histocompatibility complex heterozygote advantage and widespread bacterial infections in populations of Chinook salmon (*Oncorhynchus tshawytscha*). Molecular Ecology, **18**, 4716-4729.

Fangue NA, Hofmeister M, Schulte PM. (2006) Intraspecific variation in thermal tolerance and heat shock gene expression in the common killifish, *Fundulus heteroclitus*. Journal of Experimental Biology, **209**, 2859-287.

Fay JC, Wittkopp PJ (2008) Evaluating the role of natural selection in the evolution of gene regulation. Heredity, **100**, 191-199.

Fevolden SE, Røed KH, Fjalestad KT (2002) Selection response of cortisol and lysozyme in rainbow trout and correlation to growth. Aquaculture, **205**, 61-75.

Fraser DJ, Bernatchez L (2001) Adaptive evolutionary conservation: towards a unified concept for defining conservation units. Molecular Ecology, **10**, 2741-2752.

Fraser DJ, Weir LK, Bernatchez L, Hansen MM, Taylor EB (2011) Extent and scale of local adaptation in salmonid fishes: review and meta-analysis. Heredity, **106**, 404-420.

Fusek M, Větvička V (2005) Dual role of cathepsin D: ligand and protease. Biomedical Papers, **149**, 43-50.

Garcia de Leaniz C, Fleming IA, Einum S, Verspoor E, Jordan WC, Consuegra S, Aubin-Horth N, Lajus D, Letcher H, Youngson AF, Webb JH, Vøllestad LA, Villanueva B, Ferguson A, Quinn TP (2007) A critical review of adaptive genetic variation in Atlantic salmon: implications for conservation. Biological Reviews, **82**, 173-211.

Glover KA, Grimholt U, Bakke HG, Nilsen F, Storset A, Skaala Ã (2007) Major histocompatibility complex (MHC) variation and susceptibility to the sea louse

Lepeophtheirus salmonis in Atlantic salmon *Salmo salar*. Diseases of Aquatic Organisms, **76**, 57-65.

Harvell CD, Mitchell CE, Ward JR, Altizer S, Dobson AP, Ostfeld RS, Samuel MD (2002) Climate warming and disease risks for terrestrial and marine biota. Science, **296**, 2158-2162.

Healy TM, Tymchuk WE, Osborne EJ, Schulte PM (2010) Heat shock response of killifish (*Fundulus heteroclitus*): candidate gene and heterologous microarray approaches. Physiological Genomics, **41**, 171-184.

Holt RA, Amandi A, Rohovec JS, Fryer JL (1989) Relation of water temperature to bacterial cold-water disease in coho salmon, chinook salmon, and rainbow trout. Journal of Aquatic Animal Health, **1**, 94-101.

Hullar MA, Kaplan LA, Stahl DA (2006) Recurring Seasonal Dynamics of Microbial Communities in Stream Habitats. Applied and Environmental Microbiology, **72**, 713-722.

Jeukens J, Bittner D, Knudsen R, Bernatchez L (2009) Candidate Genes and Adaptive Radiation: Insights from Transcriptional Adaptation to the Limnetic Niche among Coregonine Fishes (*Coregonus* spp., Salmonidae) Molecular Biology and Evolution, **26**,155-166.

Johnson KA, Flynn JK, Amend DF (1982) Duration of immunity in salmonids vaccinated by direct immersion with *Yersinia ruckeri* and *Vibrio anguillarum* bacterins. Journal of Fish Diseases, **5**, 207-213.

Jonsson B, Forseth T, Jensen AJ, Næsje TF (2001) Thermal performance of juvenile Atlantic salmon, *Salmo salar* L. Functional Ecology, **15**, 701-711.

Kawecki TJ, Ebert D (2004) Conceptual issues in local adaptation. Ecology Letters, **7**, 1225-1241.

Koehler RA (2010) Adult distribution and the effects of dispersal and genetic drift on population genetic structure of resident rainbow trout (*Oncorhynchus mykiss*) in Babine Lake, British Columbia. M.Sc. Thesis, University of Windsor. 1-89.

Lorenzen E, Dalsgaard I, Bernardet JF (1997) Characterization of isolates of Flavobacterium psychrophilum associated with coldwater disease or rainbow trout fry syndrome I: phenotypic and genomic studies. Diseases of Aquatic Organisms, **31**, 197-208.

McVicar AH, Oliver G, Traxler GS, Jones S, Kieser D, MacKinnon A-M (2006) Cultured and wild fish disease interactions in the Canadian marine environment. In: A scientific

review of the potential environmental effects of aquaculture in aquatic ecosystems - volume 4. Canadian Technical Report on Fisheries and Aquatic Sciences 2450, 59-95.

Medzhitov R, Janeway CA (1997) Innate immunity: the virtues of a nonclonal system of recognition. Cell, **91**, 295-298.

Miller KM, Li S, Kaukinen KH, Ginther N, Hammill E, Curtis JMR, Patterson DA, Sierocinski T, Donnison L, Pavlidis P, Hinch SG, Hruska KA, Cooke SJ, English KK, Farrell AP (2011) Genomic signatures predict migration and spawning failure in wild Canadian salmon. Science, **331**, 214-217.

Mommsen TP, Vijayan MM, Moon TW (1999) Cortisol in teleost: dynamics, mechanisms of action, and metabolic regulation. Reviews in Fish Biology and Fisheries, **9**, 211-268.

Normandeau E, Hutchings JA, Fraser DJ, Bernatchez L (2009) Population-specific gene expression responses to hybridization between farm and wild Atlantic salmon. Evolutionary Applications, **2**, 489-503.

Ortega VA, Renner KJ, Bernier NJ (2006) Appetite-suppressing effects of ammonia exposure in rainbow trout associated with regional and temporal activation of brain monoaminergic and CRF systems. Journal of Experimental Biology, **208**, 1855-1866.

Pederson AB, Babayan SA (2011) Wild Immunology. Molecular Ecology, **20**, 872-880.

Pilkis SJ, Granner DK (1992) Molecular physiology of the regulation of hepatic gluconeogenesis and glycolysis. Annual Review of Physiology, **54**, 885-909.

Podrabsky JE, Somero GN (2004) Changes in gene expression associated with acclimation to constant temperatures and fluctuating daily temperatures in an annual killifish *Austrofundulus limnaeus*. Journal of Experimental Biology, **207**, 2237-2254.

Quinn TP (2005) The Behavior and Ecology of Pacific Salmon and Trout. University of Washington Press, Seattle, WA.

R Development Core Team (2011) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.

Raida MK, Buchmann K (2008) Development of adaptive immunity in rainbow trout, *Oncorhynchus mykiss* (Walbaum) surviving an infection with *Yersinia ruckeri*. Fish and Shellfish Immunology, **25**, 533-541.

Raida MK, Buchmann K (2009) Innate immune response in rainbow trout (*Oncorhynchus mykiss*) against primary and secondary infections with *Yersinia ruckeri* O1. Developmental and Comparative Immunology, **33**, 35-45.

Ramakers C, Ruijter JM, Lekanne Deprez RH, Moorman AFM (2003) Assumption-free analysis of quantitative real-time polymerase chain reaction (PCR) data. Neuroscience Letters 339, 62-66.

Robertson, B (2006) The interferon system of teleost fish. Fish and Shellfish Immunology, **20**, 172-191.

Scapigliati G, Buonocore F, Mazzini M (2006) Biological activity of cytokines: An evolutionary perspective. Current Pharmaceutical Design, **12**, 3071-3081.

Schluter D (2000) The Ecology of Adaptive Radiations. Oxford University Press, Oxford, UK.

Secombes CJ, Hardie LJ, Daniels G (1996) Cytokines in fish: an update. Fish and Shellfish Immunology, **6**, 291-304.

St-Cyr J, Derome N, Bernatchez L (2008) The transcriptomics of life-history trade-offs in whitefish species pairs (*Coregonus* sp.). Molecular Ecology, **17**, 1850-1870.

Taylor EB (1991) A Review of Local Adaptation in Salmonidae, with Particular Reference to Pacific and Atlantic Salmon. Aquaculture, **98**, 185-207.

Tonteri A, Vasemägi A, Lumme J, Primmer CR (2010) Beyond MHC: signals of elevated selection pressure on Atlantic salmon (*Salmo salar*) immune-relevant loci. Molecular Ecology, **19**, 1273-1282.

Tuomi JM, Voorbraak F, Jones DL, Ruijter JM (2010) Bias in the Cq value observed with hydrolysis probe based quantitative PCR can be corrected with the estimated PCR efficiency value. Methods, **50**, 313-322.

Turner SM, Faisal M, DeWoody JA (2007) Zygosity at the major histocompatibility class IIB locus predicts susceptibility to Renibacterium salmoninarum in Atlantic salmon (*Salmo salar* L.). Animal Genetics, **38**, 517-519.

Tymchuk W, O'Reilly PT, Bittman J, MacDonald D, Schulte P (2010) Conservation genomics of Atlantic salmon: variation in gene expression between and within regions of the Bay of Fundy. Molecular Ecology, **19**, 1842-1859.

Wang Q, Garrity GM, Tiedje JM, Cole JR (2007) Naïve Bayesian Classifier for Rapid Assignment of rRNA Sequences into the New Bacterial Taxonomy. Applied and Environmental Microbiology, **73**, 5261-5267.

Whitehead A, Crawford DL (2006) Neutral and adaptive variation in gene expression. Proceeding of the National Academy of Sciences USA, **103**, 5425-5430.

Whyte SK (2007) The innate immune response of finfish - A review of current knowledge. Fish and Shellfish Immunology, **23**, 1127-1151.

Wiseman S, Osachoff H, Basset E, Malhotra J, Bruno J, VanAggelen G, Mommsen TP, Vijayan MM (2007) Gene expression in the liver during recovery from an acute stressor in rainbow trout. Comparative Biochemistry and Physiology D, **2**, 234-244.

Wynne JW, Cook MT, Nowak BE, Elliot NG (2007) Major histocompatibility polymorphism associated with resistance towards amoebic gill diesease in Atlantic salmon (*Salmo salar* L.). Fish and Shellfish Immunology, **22**, 707-717.

CHAPTER III

THE RELATIVE CONTRIBUTION OF DRIFT AND SELECTION TO GENETIC DIVERGENCE AMONG BABINE LAKE TRIBUTARY POPULATIONS OF JUVENILE RAINBOW TROUT

Introduction

Genetic population structure arises when gene flow is reduced and populations begin to evolve independent of one another. The two major forces that drive divergence among populations are genetic drift and natural selection. Neutral markers (e.g. microsatellite DNA) have been used to define population structure, and thus evolutionarily significant units (ESUs) and management units for species with the goal of maximizing adaptive genetic variation within populations (e.g. Fraser and Bernatchez 2001, Winans et al 2004, Beacham et al 2004). The development of highly variable markers and drastic increases in throughput has allowed population structure to be defined at increasingly fine scales (e.g. Pearce et al 2007, Narum et al 2008, Wellband et al 2012); however, the degree to which population structure reflects adaptive differences among populations remains largely unaddressed. Indeed, the existence of population structure may allow, or reflect, the evolution of local adaptions by natural selection, but it does not indicate that it has occurred (Kaweki and Ebert 2004). A critical understanding of the processes governing population divergence requires knowledge of the role selection plays in determining divergence among populations at functional loci.

Pacific salmonids exhibit an astonishing degree of variation for many life history traits (Groot and Margolis 1991; Quinn 2005). The basis of this variation is believed to be the result of selection for traits that maximize fitness for individuals spawning in specific rivers or locations within rivers. This process, known as local adaptation, is often invoked

to explain differences in life history traits, for example: run timing, juvenile rearing strategy, morphology and developmental rates (reviewed by Taylor 1991 and Garcia de Leaniz et al 2007). Despite their long distance migrations, adult salmon have specific homing behaviors (Quinn 2005) that facilitate the formation of local populations and limits gene flow among populations. Population structure within a species across heterogeneous environments provides some of the essential conditions necessary for local adaptations to develop (Kaweki and Ebert 2004). The evolution of structured salmonid populations is a complex interaction of gene flow (low rates of straying by spawning adults), genetic drift (small effective population sizes) and forces of selection (heterogeneous environments across species' ranges). As a result, local adaptions appear to be context, trait and population specific (Fraser et al 2011) and salmon are a perfect study system for investigating the relative roles of drift and selection in the determination of population structure.

The comparison of variation within and among populations using neutral traits (drift) versus functional traits (drift and selection) is a powerful approach for providing evidence of local adaptation (Whitlock 2008) as well as disentangling the relative roles of evolutionary forces in population divergence. Divergence estimates based on additive genetic variance (Q_{ST}) or total phenotypic variance (P_{ST}) for functional traits can be directly compared with neutral genetic divergence (F_{ST}) and inferences can be made about the magnitude and mode of selection affecting those traits (Whitlock 2008). In salmonids, comparisons of Q_{ST} and F_{ST} have been used to infer diversifying selection acting on growth related traits in Coho salmon, *Oncorhynchus kisutch*, (McClelland and Naish 2007) and growth and survival traits in grayling, *Thymallus thymallus* (Koskinen et

al 2002). A promising new approach to the study of functional population divergence is the use of transcriptomics. Population-specific gene transcription profiles have been demonstrated for a variety of fish species including Atlantic salmon, *Salmo salar*, (Tymchuk et al 2010) and killifish, *Fundulus heteroclitus*, (Whitehead and Crawford 2006). Q_{ST} has even been used on gene transcription data to examine functional divergence among populations of Atlantic salmon, *Salmo salar* (Roberge et al 2007) and steelhead trout, *Oncorhynchus mykiss* (Aykanat et al 2011). This approach holds promise for dissecting the relative contribution of drift and selection to population divergence at functional traits.

Populations of resident rainbow trout (*Oncorhynchus mykiss*) that spawn in tributary creeks and rivers of Babine Lake, British Columbia are genetically structured based on microsatellite population genetic analyses, indicating reduced gene flow amongst geographically proximate and physically connected populations (Koehler 2010). The physical attributes of tributary streams to Babine Lake vary from large stable year-round systems to small dynamic systems that experience high flows during spring freshets and low or negligible flows during late summer and fall (Bustard 1989). Conditions that promote local adaptations and thus natural selection driving population divergence are present for this system. In addition, local adaptation of juvenile rainbow trout mediated by transcription at candidate genes has been demonstrated among Babine Lake tributary populations (Chapter 2). Here we investigate the relative contribution of drift and selection to the observed population divergence of juvenile Babine Lake rainbow trout using microarray technology to assay transcription at functionally relevant genes. We compare the levels of transcriptional divergence to estimates from neutral loci

and provide evidence that despite the influence of drift on population divergence at functional traits, selection also plays an important role in explaining population divergence.

Methods

Sampling sites and protocol

We sampled six tributaries of Babine Lake (Figure 3.1) known to have rainbow trout spawning populations (Bustard 1989). Tributaries were chosen to represent the geographic extent of resident rainbow trout producing watersheds, a variety of stream environmental conditions, as well as a range of genetic distances among rainbow trout populations (Koehler 2010). Approximately 50 young-of-the-year rainbow trout (*Oncorhynchus mykiss* Walbaum) were collected from each tributary by dip netting and electroshocking (Smith-Root BP-15 backpack shocker). Fish were transferred to the Department of Fisheries and Oceans' Fulton River Spawning Channel facility (2 – 6 hours travel time) on ice in heavy plastic bags (60 x 120 cm) containing ambient water from their tributary bubbled with oxygen. Tributary populations were held separated in common conditions in a 10' round tank with water flow-through from Fulton Lake (15 ± 0.5 °C). Fish were held for 5 days under starvation to acclimate to hatchery conditions and recover from the capture and transportation stress. Several fish from Tachek Creek died after transfer; however, the presence of many dead fish at the Tachek Creek sampling location indicated that this population was likely chronically stressed prior to sampling.

Challenge protocols

The experimental samples used in this study come from the same challenges as the samples of Chapter 2 but use a different subset of 4 fish from each population for each challenge. Briefly, the immune challenge consisted of a one-minute incubation in a 10% Vibrogen 2 vaccine bath containing formalin-inactivated cultures of *Vibrio anguillarum* serotypes I and II and *Vibrio ordalii* (Novartis Animal Health, Mississauga, Ontario). The temperature challenge consisted of a one hour incubation in a water bath raised 5 °C above ambient to 20 ± 0.5 °C. This water temperature was chosen to cause metabolic stress but not exceed the thermal maximum for rainbow trout. Fry were returned to their holding tank following the challenge and allowed to recover for 24 hours before sampling of tissues occurred. Control fish (t = 0) were sampled directly from the holding tanks. These individuals allow us to assess among-population differences in resting state as well as challenge-induced transcription. An overdose solution of clove oil (250 ppm) was used to humanely euthanize all fish. Gill tissues were dissected immediately and preserved in RNAlater at 4 °C. Samples were frozen at -20 °C within 5 days and stored at that temperature until further analysis. Caudal fin clips were also taken and preserved in 95% ethanol for genotype analysis.

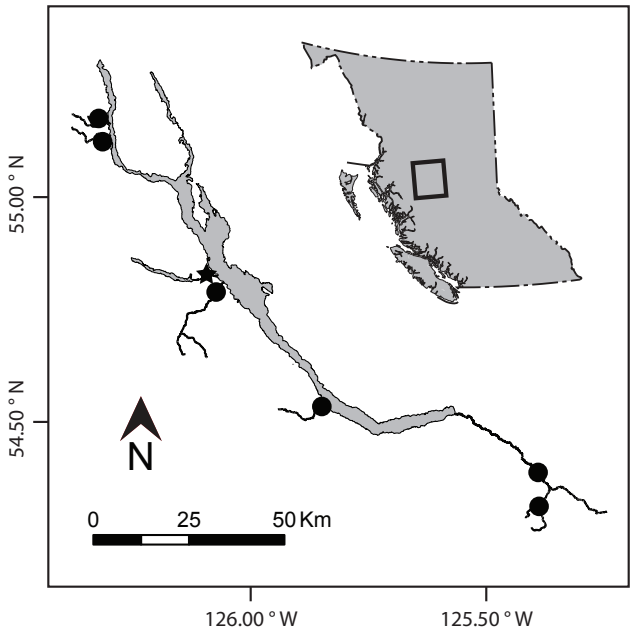

Figure 3.1: Map of Babine Lake, BC with sampling sites (filled circles) and Department of Fisheries and Oceans Fulton River facility (filled star) where challenge experiments were conducted.

Microsatellite genotype analysis

DNA was extracted from fin clips for 30 individuals from each tributary population using a salt-based extraction protocol (Elphinstone et al 2003). Individuals were genotyped at 8 microsatellite loci (Table 3.1). Polymerase chain reaction was used to amplify microsatellites in a 12.5 uL reaction containing 1X reaction buffer (10 mM Tris-HCl (pH 8.3), 50 mM KCl), 2 mM each dNTP, 40 uM dye-labeled primers, 0.125 U AmpliTaq DNA polymerase (Applied Biosystems) and 0.5 uL of DNA. PCR fragments were analyzed using a Li-Cor 4300 DNA analyzer and alleles called with Gene ImagIR software (Scanalytics). Departures from Hardy-Weinberg equilibrium and linkage disequilibrium were tested for using 10000 permutations in GenePop 4.0 (Raymond and Rousset 1995, Rousset 2008) and significance was assessed using sequential Bonferroni

correction (Rice 1989). Weir and Cockerham's (1984) unbiased estimator of F_{ST} was used to assess population differentiation in MSA 4.0 (Dieringer and Schlötterer 2003) and significance was tested by bootstrapping 10000 replicates. The distribution of F_{ST} values has been shown to approximate a chi-squared distribution with ($n_{populations} - 1$) degrees of freedom (Lewontin and Krakauer 1973) and this assumption has been shown to be robust for a wide variety of population structure models (Beaumont and Nichols 1996). Given the number of genes we analyzed from the microarray (see below) and the subsequent risk of false positives we calculated a range of confidence intervals (95, 99.9 and 99.95%) for F_{ST} using the Fdist2 program (Beaumont and Nichols 1996) and the average heterozygosity for all loci. Finally, we tested for isolation by distance using a Mantel test to correlate linearized pairwise genetic distance ($F_{ST}/1\text{-}F_{ST}$) and linear water distance between sites measured using digital 1:250000 scale topographic maps.

Microarray design & printing

We used a functionally annotated custom probe set (367 50 base oligomer probes) developed for studying stress in salmonids (http://dl.dropbox.com/u/44305264/Chinook Microarray.zip). The genes in this probe set were chosen to represent major metabolic pathways, genes involved in both innate and adaptive immune responses, xenobiotic processing as well as cell structure and genes widely used as endogenous controls in quantitative PCR studies. Probes were spotted onto poly-L-lysine coated slides (Thermo Scientific) using a SpotArray 24 microarray printing system (Perkin Elmer) equipped with Stealth Micro spotting pins (ArrayIt). Probes were printed in triplicate within each array and the array itself was replicated three times per slide. Following printing, slides

were cross-linked to the slide with UV light and blocked with succinate anhydride following Massimi et al (2002).

RNA extraction and cDNA synthesis

Gill tissue was homogenized in 0.8 mL of TRIZOL (Invitrogen) and total RNA extracted following the method of Chomczynski and Sacchi (1987). Quality of total RNA was assessed by the presence of clear 28S and 18S rRNA bands using gel-electrophoresis. Total RNA was assessed for purity and concentration using UV spectrophotometry in a Victor 3V plate reader (Perkin Elmer). All total RNA preparations had purity values of 1.9-2.1 (A260/A280). Total RNA (10 ug) was reverse transcribed into complementary DNA (cDNA) using anchored oligo dT primers (2.5 ug, Invitrogen) in a reaction containing 1X RT buffer (50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$), 5 mM DTT, 400 U of Superscript III (Invitrogen), 40 U RNaseOUT (Invitrogen) and dNTPs including amino-allyl and amino-hexyl modified nucleotides (Invitrogen). Reactions were incubated at 46 °C for 3 hours and were terminated by adding 1 uL 0.5 M EDTA and heating at 95 °C for 3 minutes. RNA was degraded by adding 15 uL of 1M NaOH and heating at 70 °C for 10 minutes and neutralized with 15 uL of 1M HCl. cDNA was precipitated overnight in a solution of 0.3 M sodium acetate and 75% ethanol at -20 °C.

cDNA labeling and microarray hybridization

Amino-allyl and amino-hexyl modified cDNA was labeled with either Alexafluor 555 or 647 (30 ug, Invitrogen) in a freshly prepared coupling buffer (0.3M $NaHCO_3$, pH

9.0) in the dark for two hours. Dyes were assigned randomly among individuals but at equal proportions within populations and treatment groups. Labeled cDNA was purified using PureLink PCR clean-up system (Invitrogen) following the manufacturers directions except for elution twice into 40 uL of 10 mM KPO_4 (pH 8.5). Labeled cDNA was hybridized to a custom low-density microarray for studying stress in salmonids in a 2X buffer (25% HiDi formamide (Applied Biosystems), 4X SSPE, 0.1% SDS, 4X Denhardt's solution) in the dark for 16 hours at 42 °C. Slides were washed once in 2X SSC / 0.1% SDS at 42 °C for 3 minutes followed by 2 minute washes once each in 2X SSC / 0.1% SDS, 1X SSC and 0.1X SSC at room temperature. Slides were scanned immediately using a ScanArray Express scanner (Perkin Elmer) and quantified using ScanArray Express Microarray Analysis System software version 4.0 (Perkin Elmer).

Data analysis

We analyzed the microarray data as a one-color (channel) experiment. Individual spots were excluded if their signal to noise ratio was less than two. Spots were background corrected using the *'normexp'* algorithm with an offset of 50 following Ritchie et al (2007) in the limma package (Smyth 2005) in the statistical software R (R Development Core Team 2011). Between-array normalization was performed using quantile normalization (Smyth and Speed 2003). We used linear mixed-effects models implemented in the R package lme4 (Bates et al 2011) to first test for differential expression of genes within each population in response to the challenges. For each gene we partitioned the variance observed in signal intensity using the following mixed model:

$$x_{aijkl} = \mu + T_a + I_j + B_{k(j)} + e_{ajkl}$$

Here, x_{ajkl} is the normalized intensity averaged over replicate spots (l) in the k^{th} block, nested within the j^{th} individual, all as random effects in the model and with the challenge (T_a) as a fixed effect. Significance of differential expression was determined using a likelihood ratio test between two models: one with and one without the challenge effect (T_a) included. Parameters were estimated using maximum likelihood (ML) to allow quantification of the effect of changes in fixed factors among the models.

To estimate divergence among populations for transcription at the functional genes we assayed, we fit three separate models (one for each treatment group) for each population. The models were similar to the linear mixed model above, but without the treatment term. These models were fitted using restricted maximum likelihood to provide an unbiased estimate of model parameters. We then used the estimated parameters from those models as priors to calculate the highest probability density (HPD) for the parameters using Markov Chain Monte Carlo simulation (1000 reps) in the R package languageR (Baayen 2011, Baayen et al 2008). Median HPD values were used as parameter estimates for the variance components to calculate phenotypic (or functional) divergence estimates. Variance explained by the random population term was taken as the among-population variance component (σ^2_{GB}) while the residual variance was taken as the within-population variance component (σ^2_{GW}). The measure Q_{ST}, strictly speaking, implies the additive component of genetic variation among groups. In this study we cannot separate possible environmental influences on phenotype, thus we denote our phenotypic (transcriptional) divergence as 'P_{ST}', following Whitlock (2008). P_{ST} was calculated using the formula $P_{ST} = \sigma^2_{GB} / (\sigma^2_{GB} + 2\ \sigma^2_{GW})$ following Whitlock (2008).

Loci were assigned to one of three groups: 1) those with P_{ST} less than the lower bound of the confidence interval for F_{ST} (indicative of stabilizing selection), 2) those within the confidence interval for F_{ST} (indicative of neutral drift) and 3) those that exceed the upper bound of the confidence interval for F_{ST} (indicative of divergent selection).

We tested for biases in patterns of divergent selection among functional groups of genes by using gene ontology (GO) information for the annotated genes on our array. We first classified the biological function (metabolic function, immune response, or other) of each gene using the BLAST mapping and annotation functions in the software package Blast2GO (Conesa et al 2005). We then used a Kruskal-Wallis test to test for biases in the rank position of P_{ST} divergence for metabolic genes in the temperature challenge and immune genes in the immune challenge. If selection on metabolic (or immune) genes were driving the divergence among populations, then we would expect those functional groups to have higher P_{ST} values relative to the other functional groups. Finally we tested for a pattern of isolation by distance among pairwise P_{ST} values and geographic distance using Mantel tests. If gene transcription differences among populations are a result of genetic drift then we expect patterns of divergence to correlate with geographic distance because F_{ST} for these populations follows this pattern (Koehler 2010). Pairwise P_{ST} values were calculated for each gene in each pairwise comparison of populations using the same linear model as previously used for P_{ST} calculations except each model only included the data for the populations comparison of interest.

Results

Genotyping

Tests for departures from Hardy-Weinberg equilibrium (HWE) detected 19 (40%) loci by population comparisons that deviated from expectations after Bonferroni correction. One population had 7 out of 8 loci that departed from HWE expectations that were responsible for many of the HWE departures. This was likely the result of non-random sampling as these individuals had to be netted from small pools in a nearly dry Tachek Creek. Otherwise, there were no loci in particular that accounted for departures from HWE expectations. Tests for linkage disequilibrium identified 10 (6%) of loci pairs by population that showed evidence of linkage. All loci demonstrated highly significant ($p < 0.001$) population structure following permutation tests. Estimates for global F_{ST} ranged from 0.025 (Omy325) to 0.082 (OtsG243) with an overall average value of 0.051 (Table 3.1). The 95% confidence interval for mean F_{ST} was determined to be 0.017 – 0.080, the 99.9% interval was 0.006 – 0.126 and the 99.95% interval was 0.004 – 0.134. Values of P_{ST} subsequently determined to be outside this interval indicate divergence among populations that cannot be explained solely by genetic drift. The Mantel test indicated that genetic differentiation at these microsatellite (neutral) loci follows a pattern of isolation by distance ($R^2 = 0.53$, $p = 0.05$).

Table 3.1: Microsatellites used to determine neutral genetic population structure among six Babine Lake tributary populations of rainbow trout. F_{ST} – Weir and Cockerham (1984) theta, T_m – annealing temperature for PCR reactions.

Loci	F_{ST}	T_m (°C)	Reference
Omy325	0.025	58	Olsen et al 1996
Ots4	0.047	58	Banks et al 1999
RT191	0.045	63	Spies et al 2005
RT212	0.058	64	Spies et al 2006
OtsG83b	0.066	56	Williamson et al 2002
OtsG249	0.051	64	Williamson et al 2003
OtsG243	0.082	52	Williamson et al 2004
OtsG401	0.036	62	Williamson et al 2005

Microarray

We analyzed between 72 – 136 genes per population for transcriptional response to stress in the temperature challenge and between 42 – 185 genes per population for transcriptional response to the immune challenges (Appendix A). The proportion of genes differentially expressed among populations ranged from 2-3% up to 20-22% of genes analyzed (Figures 3.2 and 3.3).

P_{ST} values for divergence among populations ranged from 0.029 – 0.30 (101 genes) among the temperature challenge group, 0.037 – 0.29 (49 genes) among the immune challenge group and 0.029 – 0.30 (86 genes) among the control group (Figure 3.4, Appendix B). As a result, no genes in any treatment group appeared to be under stabilizing selection. For the temperature challenged and control group P_{ST} we used a conservative threshold of the 99.95% confidence interval of F_{ST} to assess which genes may be evolving by diversifying selection. For the immune challenged P_{ST} we used a threshold of the 99.9% confidence interval of F_{ST}. These thresholds were chosen to account for the possibility of false positives in P_{ST} calculations and are analogous to Bonferroni corrections for the number of loci analyzed.

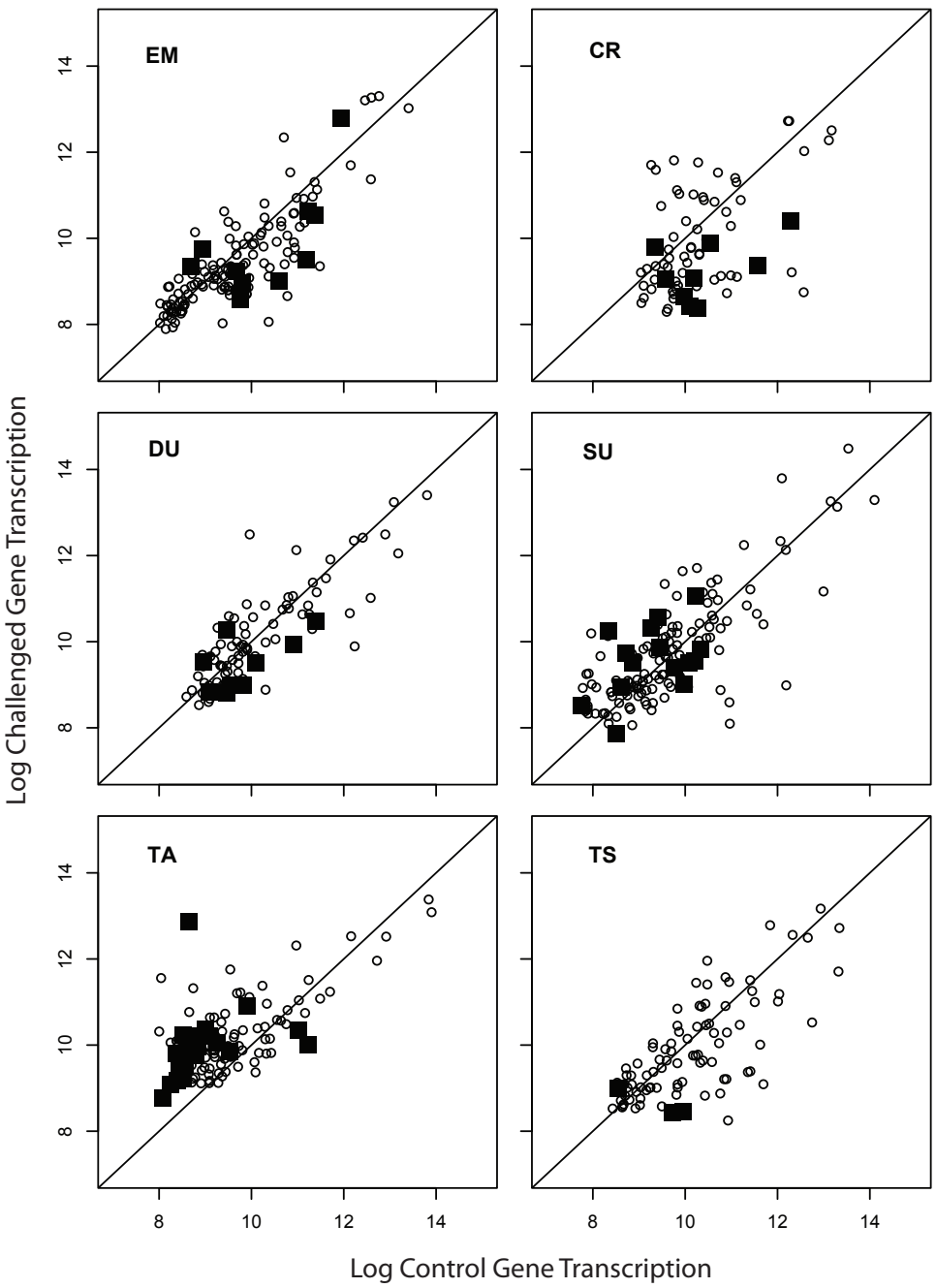

Figure 3.2: Transcriptional response to metabolic/temperature challenge for juvenile populations from six Babine Lake tributaries (EM – 11 Mile Creek, CR – Cross Creek, DU – Duncan Creek, SU – Sutherland River, TA –Tachek Creek, TS – Tsak Creek). Transcription data for genes under control conditions is plotted against transcription data under challenged conditions. Differentially transcribed genes are shown as solid squares.

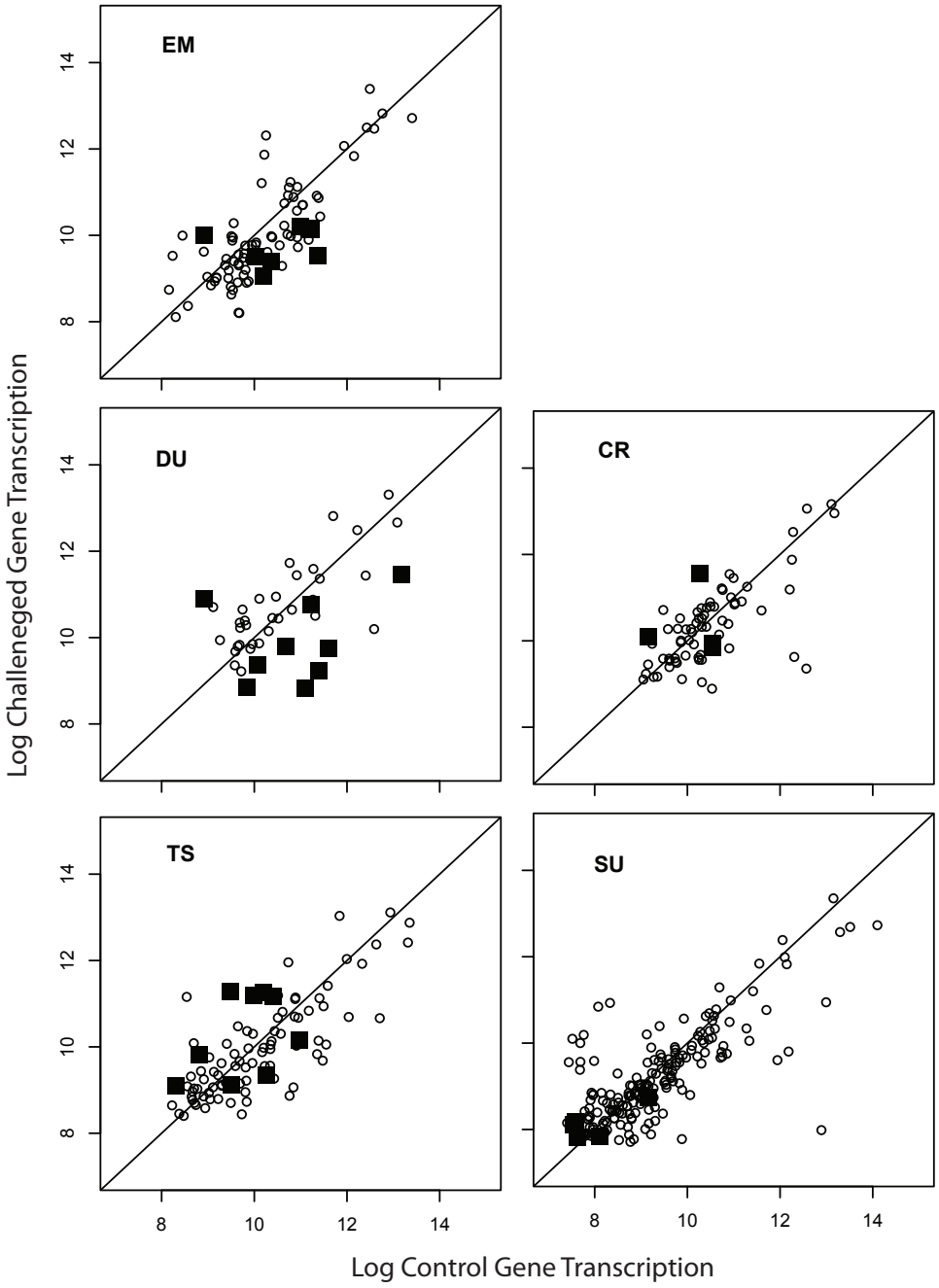

Figure 3.3: Transcriptional response to immune challenge for juvenile populations from five Babine Lake tributaries (EM – 11 Mile Creek, CR – Cross Creek, DU – Duncan Creek, SU – Sutherland River, TS – Tsak Creek, Tachek Creek samples were not analyzed due to insufficient quantities of RNA in the immune challenged group). Transcription data for genes under control conditions is plotted against raw transcription data under challenged conditions. Differentially transcribed genes are shown as solid squares.

We found the divergence estimate to be consistent with divergent selection for 38 (78%), 64 (63%), and 56 (65%) genes in each of the immune challenged, temperature challenge and control states respectively. This suggests that selection has contributed to transcriptional divergence among populations for these genes. The remaining 11, 37, and 30 genes analyzed for each treatment respectively have divergence estimates that are consistent with evolution by drift.

Results of the Kruskal-Wallis test indicated that neither metabolic genes ($X^2_{(df = 1)} = 2.52$, $p = 0.11$) nor immune genes ($X^2_{(df = 1)} = 0.92$, $p = 0.34$) more highly divergent (i.e. higher rank of P_{ST} values) that random expectations. The divergence in gene transcription among populations follows a pattern of isolation by distance (mantel test $p < 0.05$) for only 2 (3.6%), 6 (8.3%) and 1 (2.3%) genes in the control, temperature challenge and immune challenge groups respectively (Figure 3.4, Appendix B).

Discussion

We have demonstrated that population divergence in gene transcription is mediated by both drift and selection for Babine Lake tributary juvenile rainbow trout. Phenotypic divergence (Q_{ST}) has previously been used to identify gene transcription under selection (e.g. Roberge et al 2007, Aykanat et al 2011). Roberge et al (2007) utilized a transcriptome scan approach with the assumption that most loci were under stabilizing selection and identified 3% of genes putatively evolving by divergent selection. By comparison, their Q_{ST} estimates for all genes they analyzed were, on average, much lower than those presented here. However when considering only the genes identified in Roberge et al (2007) as divergent, the range of Q_{ST} values (0.07 –

0.19) was comparable to the P_{ST} values we present. Q_{ST} or P_{ST} values for gene transcription that exceed the range of F_{ST} values for neutral loci indicate that transcription of these genes are more divergent than expected based on genetic drift alone (Whitlock 2008). This implies that natural selection is driving the development of local adaptation for transcriptional traits.

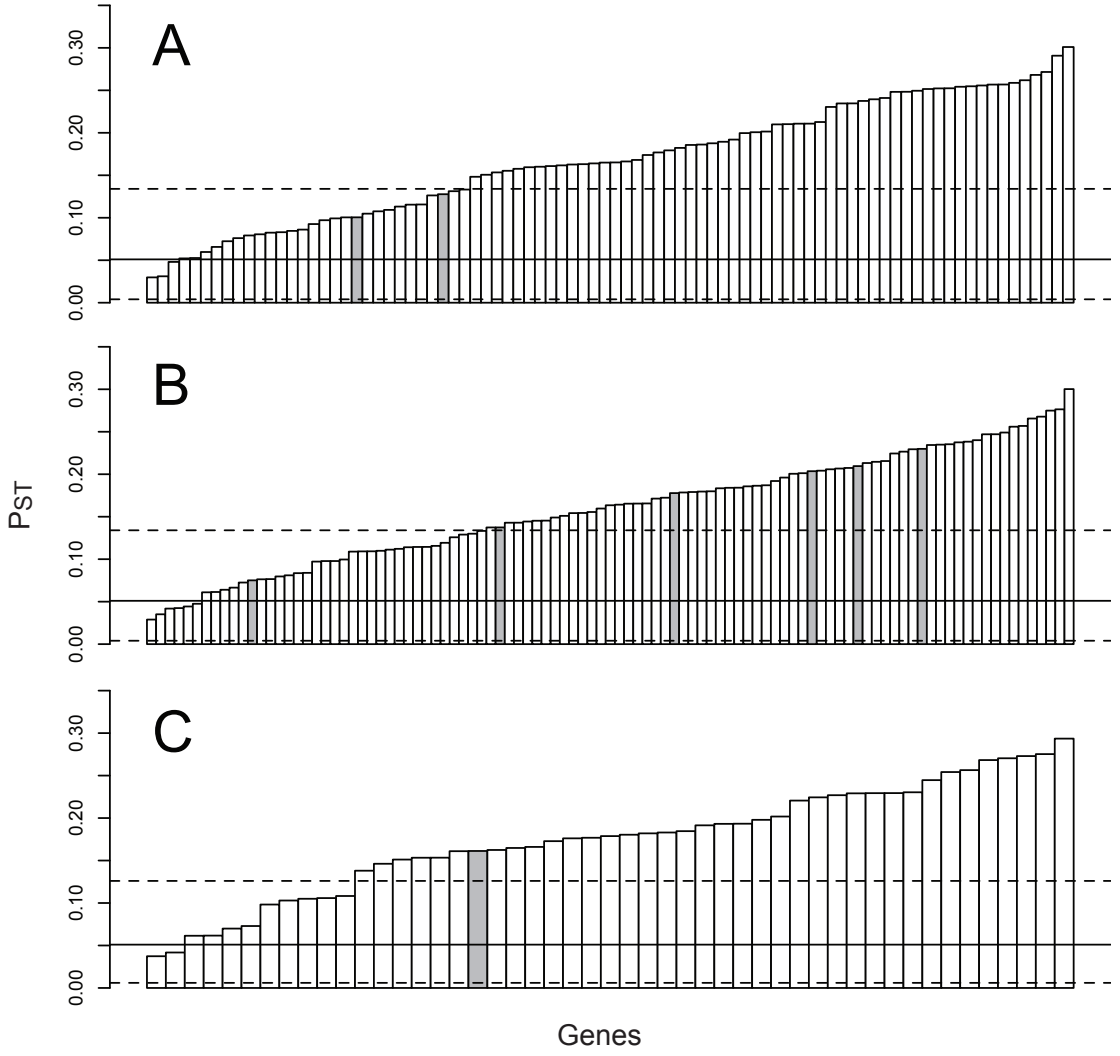

Figure 3.4: Bar chart showing P_{ST} for transcription of genes arranged in increasing order for fish under; control (A), temperature challenged (B), and immune challenged (C) conditions. The solid line represents the mean F_{ST} from neutral microsatellite markers, while the dashed line represents the 99.9% confidence interval (immune challenge) or 99.95% confidence interval (control and temperature challenge) for F_{ST}. Shaded bars indicate gene transcription divergence that follows a relationship of isolation by distance.

Population-specific transcription profiles have also been demonstrated in comparisons among genetically divergent populations of wild Atlantic salmon (Tymchuk et al 2010). Tymchuk et al (2010) found that hierarchical clustering of transcription profiles reliably separated populations of Atlantic salmon from the Bay of Fundy and, similar to our results, reported no significant correlation between transcriptional divergence and neutral genetic divergence. That lack of correlation likely reflects the different evolutionary pressures and histories of transcription versus neutral loci. The direction and strength of selection on transcriptional traits are likely to reflect the environmental conditions of habitats specific to each population (Chapter 2). As such, the hierarchical clustering of transcription traits better reflects environmental differences among populations, and thus should not vary with geographic distance as neutral loci often do. Our results showed that, for the majority of genes, transcription does not follow a pattern of isolation by distance. Since isolation by distance is predicated upon genetic drift – gene flow equilibrium, our transcriptional divergence pattern is not likely to be due to evolution by drift. These results provide further evidence that the patterns of divergence we have demonstrated are the result of natural selection forming local adaptations for transcriptional traits in tributary populations of Babine Lake juvenile rainbow trout. It should be noted however, that a significant isolation by distance pattern may still indicate a primarily selection-based response, if the populations are situated along a gradient of environmental selection that is geographically structured (e.g. Whitehead and Crawford 2006, Bradbury et al 2010).

Transcriptional adaptation among salmonid species pairs with different energetic requirements has been demonstrated using a transcriptional trade-off model of survival

and growth related genes (Derome et al 2006, St-Cyr et al 2008). Additional transcriptional adaptations among species utilizing benthic and limnetic niches have been identified using a candidate gene approach to test hypotheses about the energetic requirements for each life history (Jeukins et al 2009). Those studies highlight the role selection can play in the evolution of transcriptional regulation of metabolic genes. While our analysis did not identify functional groups of genes that were over- or under-represented in comparisons of drift versus selection among populations, our array is enriched for genes associated with immune and metabolic functions that have previously been implicated in response to metabolic (e.g. Wiseman et al 2007) and immune challenges (e.g. Raida and Buchmann 2008). It is possible that we do not have sufficient power to detect enrichment of these groups of genes in our study: that is, we have a biased sample of genes that have a higher than random likelihood of being under selection.

A large portion (~60%) of the genes we studied appeared to be influenced by divergent selection. Our inability to partition the observed transcriptional variance into additive genetic variance component leaves the possibility that previous environmental exposure differences among the sampled populations are influencing our estimates of transcriptional divergence. However, Whitlock (2008) has suggested that such environmental effects would inflate estimates of within-population variability and thus underestimate phenotypic divergence. This means that if environmental effects are influencing our estimates we have, at worst, presented conservative estimates of population transcriptional divergence. Further to this, environmental contributions to transcriptional variation of metabolic genes in wild caught *Fundulus heteroclitus* have

been shown to not influence differences among groups (Scott et al 2009). Despite the considerable non-additive genetic variation described for transcriptional traits (Gibson and Weir 2005), other studies have demonstrated a good deal of additive genetic variation for gene transcription (Brem and Kruglyak 2005, Roberge et al 2007) suggesting the transcriptional differences we have demonstrated represent, at least for some loci, functional differences among populations indicative of local adaptation.

We have demonstrated transcriptional divergence among tributary populations of juvenile rainbow trout in Babine Lake. Comparisons of the patterns and magnitude of transcriptional divergence for genes with estimates of neutral divergence highlight the roles of both drift and selection in driving population structure at a functional level. The action of selection on the transcriptome suggests a possible genetic mechanism for the process of local adaptation and gives real weight to the significance of population structure for conservation and management of salmonid species. Population genetic structure, though often derived from neutral loci, does represent real functional (transcriptional) differences among populations that in turn reflect local adaptations of populations. This work reinforces the need to conserve salmonids at the population or tributary level to preserve this diversity and adaptive potential in functional traits.

References

Aykanat T, Thrower FP, Heath DD (2011) Rapid evolution of osmoregulatory function by modification of gene transcription in steelhead trout. Genetica, **139**, 233-242.

Baayen RH (2011) languageR: Data sets and functions with "Analyzing Linguistic Data: A practical introduction to statistics". R package version 1.4. http://CRAN.R-project.org/package=languageR

Baayen RH, Davidson DJ, Bates DM (2008) Mixed-effects modeling with crossed random effects for subjects and items. Journal of Memory and Language, **59**, 390-412.

Banks MA, Blouin MS, Baldwin BA, Rashbrook VK, Fitzgerald HA, Blankenship SM, Hedgecock D (1999) Isolation and inheritance of novel microsatellites in Chinook salmon (*Oncorhynchus tshawytscha*). Journal of Heredity, **90**, 281-288.

Bates D, Maechler M, Bolker B (2011) lme4: Linear mixed-effects models using S4 classes. R package version 0.999375-42. http://CRAN.R-project.org/package=lme4

Beacham TD, Lapointe M, Candy JR, Miller KM, Withler RE (2004) DNA in action: rapid application of DNA variation to sockeye salmon fisheries management. Conservation Genetics, **5**, 411-416.

Beaumont MA, Nichols RA (1996) Evaluating loci for use in the genetic analysis of population structure. Proceedings of the Royal Society of London B, **263**, 1619-1626.

Bradbury IR, Hubert S, Higgins B, Borza T, Bowman S, Paterson IG, Snelgrove PVR, Morris CJ, Gregory RS, Hardie DC, Hutchings JA, Ruzzante DE, Taggart CT, Bentzen P (2010) Parallel adaptive evolution of Atlantic cod on both side of the Atlantic Ocean in response to temperature. Proceeding of the Royal Society B, **277**, 3725-3734.

Brem RB, Kruglyak L (2005) The landscape of genetic complexity across 5,700 gene expression traits in yeast. Proceeding of the National Academy of Sciences USA, **102**, 1572-1577.

Bustard D (1989) Assessment of rainbow trout recruitment from streams tributary to Babine Lake. Consultant report prepared for Skeena Region Fisheries Branch, BC Ministry of Environment and Parks. Smithers, BC. 1-71.

Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Analytical Biochemistry, **162**(1), 156-159.

Conesa A, Götz S, Garcia-Gomez JM, Terol J, Talon M, Robles M (2005) Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. Bioinformatics, **21**, 3674-3676.

Derome N, Duchesne P, Bernatchez L (2006) Parallelism in gene transcription among sympatric lake whitefish (*Coregonus clupeaformis* Mitchill) ecotypes. Molecular Ecology, **15**, 1239-1249.

Dieringer D, Schlötterer C (2003) Microsatellite analyser (MSA): a platform independent analysis tool for large microsatellite data sets. Molecular Ecology Notes, **3**, 167-169.

Elphinstone MS, Hinten GN, Anderson MJ, Nock CJ (2003) An inexpensive and high-throughput procedure to extract and purify total genomic DNA for population studies. Molecular Ecology Notes, **3**, 317-320.

Fraser DJ, Bernatchez L (2001) Adaptive evolutionary conservation: towards a unified concept for defining conservation units. Molecular Ecology, **10**, 2741-2752.

Fraser DJ, Weir LK, Bernatchez L, Hansen MM, Taylor EB (2011) Extent and scale of local adaptation in salmonid fishes: review and meta-analysis. Heredity, **106**, 404-420.

Garcia de Leaniz C, Fleming IA, Einum S, Verspoor E, Jordan WC, Consuegra S, Aubin-Horth N, Lajus D, Letcher H, Youngson AF, Webb JH, Vøllestad LA, Villanueva B, Ferguson A, Quinn TP (2007) A critical review of adaptive genetic variation in Atlantic salmon: implications for conservation. Biological Reviews, **82**, 173-211.

Gibson G, Weir B (2005) The quantitative genetics of transcription. Trends in Genetics, **21**, 616-623.

Groot C, Margolis L (1991) Pacific salmon life histories. University of British Columbia Press, Vancouver.

Jeukens J, Bittner D, Knudsen R, Bernatchez L (2009) Candidate Genes and Adaptive Radiation: Insights from Transcriptional Adaptation to the Limnetic Niche among Coregonine Fishes (*Coregonus* spp., Salmonidae) Molecular Biology and Evolution, **26**,155–166.

Kawecki TJ, Ebert D (2004) Conceptual issues in local adaptation. Ecology Letters, **7**, 1225-1241.

Koehler RA (2010) Adult distribution and the effects of dispersal and genetic drift on population genetic structure of resident rainbow trout (Oncorhynchus mykiss) in Babine Lake, British Columbia. M.Sc. Thesis, University of Windsor. 1-89.

Koskinen MT, Haugen TO, Primmer CR (2002) Contemporary fisherian life-history evolution in small salmonid populations. Nature, **419**, 826-830.

Lewontin RC, Krakauer J (1973) Distribution of gene frequency as a test of the theory of the selective neutrality of polymorphisms. Genetics, **74**, 175-195.

Massimi A, Harris T, Childs G, Somerville S (2002) Printing on Glass Slides. In A Molecular Cloning Manuel: DNA Microarrays. Bowtell D, Sambrook J, eds. Cold Spring Harbor Laboratory Press Cold Spring Harbor, NY. Pp. 71-78.

McClelland EK, Naish KA (2007) Comparison of F_{ST} and Q_{ST} of growth-related traits in two populations of Coho salmon. Transactions of the American Fisheries Society, **136**, 1276-1284.

Narum SR, Zendt JS, Graves D, Sharp B (2008) Influence of landscape on resident and anadromous life history types of *Oncorhynchus mykiss*. Canadian Journal of Fisheries and Aquatic Sciences, **65**, 1013-1023.

Olsen JB, Wenburg JK, Bentzen P (1996) Semiautomated multilocus genotyping of Pacific salmon (*Oncorhynchus* spp.) using microsatellites. Molecular Marine Biology and Biotechnology, **5**, 259-272.

Pearse DE, Donohoe CJ, Garza JC (2007) Population genetics of steelhead (*Oncorhynchus mykiss*) in the Klamath River. Environmental Biology of Fishes, **80**, 377-387.

Quinn TP (2005) The Behavior and Ecology of Pacific Salmon and Trout. University of Washington Press, Seattle, WA.

R Development Core Team (2011) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL: http://www.R-project.org/.

Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity, **86**, 248-249.

Raida MK, Buchmann K (2008) Development of adaptive immunity in rainbow trout, *Oncorhynchus mykiss* (Walbaum) surviving an infection with *Yersinia ruckeri*. Fish and Shellfish Immunology, **25**, 533-541.

Rice WR (1989) Analyzing tables of statistical tests. Evolution, **43**, 223-225.

Ritchie ME, Silver J, Oshlack A, Silver J, Holmes M, Diyagama D, Holloway A, Smyth GK (2007) A comparison of background correction methods for two-colour microarrays. Bioinformatics, **23**, 2700-2707.

Roberge C, Guderly H, Bernatchez L (2007) Genomewide identification of genes under directional selection: gene transcription Q_{ST} scan in diverging Atlantic salmon subpopulations. Genetics, **177**, 1011-1022.

Rousset F (2008) GENEPOP ’007: a complete re-implementation of the GENEPOP software for Windows and Linux. Molecular Ecology Resources, **8**, 103-106.

Scott CP, Williams DA, Crawford DL (2009) The effect of genetic and environmental variation on metabolic gene expression. Molecular Ecology, **18**, 2832-2843.

Smyth GK (2005) Limma: linear models for microarray data. In: Bioinformatics and Computational Biology Solutions using R and Bioconductor. R. Gentleman, V. Carey, S. Dudoit, R. Irizarry, W. Huber (eds), Springer, New York, pages 397-420.

Smyth GK, Speed TP (2003) Normalization of cDNA microarray data. Methods, **31**, 265-273.

Spies IB, Brasier DJ, O'Reilly PTL, Seamons TR, Bentzen P (2005) Development and characterization of novel tetra-, tri-, and dinucleotide microsatellite markers in rainbow trout (*Oncorhynchus mykiss*). Molecular Ecology Notes, **5**, 278-281.

St-Cyr J, Derome N, Bernatchez L (2008) The transcriptomics of life-history trade-offs in whitefish species pairs (*Coregonus* sp.). Molecular Ecology, **17**, 1850-1870.

Taylor EB (1991) A review of local adaptation in Salmonidae, with particular reference to Pacific and Atlantic salmon. Aquaculture, **98**, 185-207.

Tymchuk W, O'Reilly PT, Bittman J, MacDonald D, Schulte P (2010) Conservation genomics of Atlantic salmon: variation in gene expression between and within regions of the Bay of Fundy. Molecular Ecology, **19**, 1842–1859.

Weir BS, Cockerham CC (1984) Estimating F-Statistics for the Analysis of Population-Structure. Evolution, **38**(6), 1358-1370.

Whitehead A, Crawford DL (2006) Neutral and adaptive variation in gene expression. Proceeding of the National Academy of Sciences USA, **103**, 5425-5430.

Whitlock MC (2008) Evolutionary inference from Q_{ST}. Molecular Ecology, **17**, 1885-1896.

Williamson KS, Cordes JF, May B (2002) Characterization of microsatellite loci in Chinook salmon (*Oncorhynchus tshawytscha*) and crossspecies amplification in other salmonids. Molecular Ecology Notes, **2**, 17–19.

Wellband KW, Atagi DY, Koehler RA, Heath DD (2012) Fine-scale population genetic structure and dispersal of juvenile steelhead in the Bulkley-Morice river, British Columbia. Transactions of the American Fisheries Society, **141**, 392-401.

Winans GA, Paquin MM, Van Doornik DM, Baker BM, Thornton P, Rawding D, Marshall A, Moran P, Kalinowski S (2004) Genetic stock identification of steelhead in the Columbia River Basin: An evaluation of different molecular markers. North American Journal of Fisheries Management, **24**(2), 672-685.

Wiseman S, Osachoff H, Basset E, Malhotra J, Bruno J, VanAggelen G, Mommsen TP, Vijayan MM (2007) Gene expression in the liver during recovery from an acute stressor in rainbow trout. Comparative Biochemistry and Physiology D, **2**, 234-244.

CHAPTER IV

CONCLUSION

The importance of local adaptations for the conservation of biodiversity cannot be overstated (Fraser and Bernatchez 2001). Local adaptations also play a critical role in initiating and reinforcing speciation processes (Schluter 2001). Salmonid species are particularly well suited for studying local adaptations due to their high natal philopatry, specific homing behaviors and tendency to form structured populations across heterogeneous habitats (Quinn 2005). Despite a considerable body of literature describing local adaptation of phenotypic traits in salmonid species (Taylor 1991, Garcia de Leaniz et al 2007), the genetic mechanisms underlying local adaptations remain poorly characterized. Evolution of gene transcription has been highlighted for the role it plays in the development of local adaptations (Bernatchez et al 2010) and shows promise for describing, at least in part, the rapid evolution of local adaptation, and possibly acclimation, in salmonid species. This thesis contributes to our knowledge of the genetic mechanisms of local adaptation by investigating divergence among transcriptional traits of genetically structured populations of rainbow trout, *Oncorhynchus mykiss*, from Babine Lake, BC.

Chapter 2 describes my utilization of a candidate gene approach to demonstrate that transcriptional differences among populations are correlated with physical and biological properties of the tributary streams in which they were spawned and reared. The relationship was stronger when individuals were challenged with either a temperature stress or an immune stress, suggesting that selection acts on both the resting state as well as induced levels of gene transcription. In Chapter 3, I examined transcription in a wider

sample of functionally important genes using microarray technology. Evidence for widespread and substantial population structure based on transcriptional variance was demonstrated for Babine Lake rainbow trout populations. The pattern of transcriptional divergence did not follow the pattern expected for neutral divergence based on microsatellite DNA, indicating that natural selection plays a major role in the process of population divergence and implying that transcriptional divergence reflects local adaptation to tributary stream conditions.

Here I discuss what I believe to be the key findings of this thesis, and explore their implications for both biodiversity conservation and the evolution of functional divergence (local adaptation). Perhaps the most obvious point is that gene transcription varies dramatically among the geographically proximate tributary populations studied in this thesis. This is reflective of the high intraspecific transcriptional variation that has previously been demonstrated in a variety of species (e.g. Oleksiak et al 2002). Those results stand in contrast to the belief that stabilizing selection governs the majority of gene transcription evolution (Bedford and Hartl 2005). Ultimately, transcriptional variance must reflect the limits of physiological tolerance; however, there is no reason to believe that population-specific transcription profiles could not evolve beyond the current physiologically tolerable range for a species, given sufficient genetic variance and strong enough selection pressure.

The larger concern for this thesis is whether the differences among populations reflect adaptation or random divergence. This question was addressed in a number of ways. First, the population-specific values for resting state transcription and response to temperature and immune challenges were correlated with tributary environmental

variables that likely reflect selective pressures for the transcription of candidate genes selected for study (Chapter 2). The demonstration of significant correlations indicates that transcriptional divergence is driven by environmental differences among the tributaries. Thus those data not only suggest that transcriptional differences are not random, but also provide a clear mechanism for the development of local adaptations mediated by evolution of gene transcription regulation. Environmental-gene transcription associations have been previously described for *Fundulus heteroclitus* along a latitudinal gradient of temperature for transcription of metabolic genes (Whitehead and Crawford 2006) and heat shock genes (Fangue et al 2006). The role of pathogen community diversity and abundance-mediated selection on the salmonid immune system is well documented (Evans and Neff 2009, Dionne et al 2009). Together those results and my findings reinforce the role of thermal regime and pathogen-mediated selection on the formation of local adaptation in fish, with modification of gene transcription being a logical and likely mechanism for that process.

The second way this thesis addressed the issue of whether transcriptional differences were random was to compare transcriptional divergence with the expected outcomes of evolution by genetic drift. To this end, transcriptional population divergence was assayed for approximately 50 – 100 genes in each of three treatment groups (control, temperature challenged and immune challenged). The magnitude of the transcriptional divergence was then compared to the distribution of divergence estimates derived from neutral microsatellite DNA markers. A large proportion of genes were determined to have transcriptional divergence that exceeded the estimates of neutral expectations. In addition, the patterns of pairwise divergence among populations were regressed with

geographic distance to test the neutral genetic theory of isolation by distance. This pattern was only demonstrated for transcriptional divergence at a handful of loci, suggesting that the majority of transcriptional divergence has evolved in a pattern not consistent with neutral genetic expectations. Other examples of population divergence in transcription inconsistent with neutral expectations have been demonstrated in *Salmo salar* (Roberge et al 2007; Tymchuk et al 2010). In those cases the most parsimonious explanation is that observed differences in gene transcription among populations reflect adaptive divergence and thus local adaptation.

Future directions

The initial goal of this study was to conduct controlled breeding experiments on adult fish from each tributary. Capturing enough adult fish proved to be logistically difficult; however, the knowledge gained from these initial efforts should allow for success in setting up future breeding experiments. These experiments would allow me to further partition transcriptional variance into heritable and environmental components that would provide more reliable estimates of population level differences in gene transcription. In addition, with controlled breeding experiments there would be the possibility of conducting reciprocal transplant experiments to assess fitness consequences of rearing in a non-natal / non-local environment and to associate fitness consequences with transcriptional patterns of particular genes.

A growing body of evidence, including the novel approaches described here, reinforces the role of gene expression processes in the formation of local adaptation in salmonids. In this regard, the literature has generally focused on gene transcription as the

primary means by which gene expression is regulated; however, other post-transcriptional processes (especially those directly affecting the transcript; e.g., RNAi, mRNA stability, alternative splicing) are known to play important roles in the translation of genetic information into phenotype (Berezikov 2011, Huntzinger and Izaurralde 2011). Given the advances in next-generation sequencing technology, evolutionary biologists are now employing techniques that were previously restricted to physiologists and immunologists working with model species and well-characterized laboratory strains (Pederson and Babayan 2011). Implementation miRNA assays within a population genetic framework (much like that done here with gene transcription) will undoubtedly provide a more complete picture of the processes governing gene expression-mediated adaptations in natural populations.

References

Bedford T, Hartl DL (2009) Optimization of gene expression by natural selection. Proceeding of the National Academy of Sciences USA, **106**, 1133-1138.

Berezikov E (2011) Evolution of microRNA diversity and regulation in animals. Nature Reviews Genetics, **12**, 846-860.

Bernatchez L, Renaut S, Whiteley AR, Derome N, Jeukins J, Landry L, Lu G, Nolte A, Ostbye K, Rogers SM, St-Cyr J (2010) On the origin of species: insights from the ecological genomics of lake whitefish. Philosophical Transactions of the Royal Society B, **365**, 1783-1800.

Dionne M, Miller KM, Dodson JJ, Bernatchez L (2009) MHC standing genetic variation and pathogen resistance in wild Atlantic salmon. Philosophical Transactions of the Royal Society B, **364**, 1555-1565.

Evans ML, Neff BD (2009) Major histocompatibility complex heterozygote advantage and widespread bacterial infections in populations of Chinook salmon (*Oncorhynchus tshawytscha*). Molecular Ecology, **18**, 4716-4729.

Fangue NA, Hofmeister M, Schulte PM. (2006) Intraspecific variation in thermal tolerance and heat shock gene expression in the common killifish, *Fundulus heteroclitus*. Journal of Experimental Biology, **209**, 2859-287.

Fraser DJ, Bernatchez L (2001) Adaptive evolutionary conservation: towards a unified concept for defining conservation units. Molecular Ecology, **10**, 2741-2752.

Garcia de Leaniz C, Fleming IA, Einum S, Verspoor E, Jordan WC, Consuegra S, Aubin-Horth N, Lajus D, Letcher H, Youngson AF, Webb JH, Vøllestad LA, Villanueva B, Ferguson A, Quinn TP (2007) A critical review of adaptive genetic variation in Atlantic salmon: implications for conservation. Biological Reviews, **82**, 173-211.

Huntzinger E, Izaurralde E (2011) Gene silencing by microRNAs: contributions of translational repression and mRNA decay. Nature Reviews Genetics, **12**, 99-110.

Oleksiak MF, Churchill GA, Crawford DL (2002) Variation in gene expression within and among natural populations. Nature Genetics, **32**, 261-266.

Pederson AB, Babayan SA (2011) Wild Immunology. Molecular Ecology, **20**, 872-880.

Quinn TP (2005) The Behavior and Ecology of Pacific Salmon and Trout. University of Washington Press, Seattle, WA.

Roberge C, Guderly H, Bernatchez L (2007) Genomewide identification of genes under directional selection: gene transcription Q_{ST} scan in diverging Atlantic salmon subpopulations. Genetics, **177**, 1011-1022.

Schluter D (2001) Ecology and the origin of species. Trends in Ecology and Evolution, **16**, 372-380.

Taylor EB (1991) A Review of Local Adaptation in Salmonidae, with Particular Reference to Pacific and Atlantic Salmon. Aquaculture, **98**, 185-207.

Tymchuk W, O'Reilly PT, Bittman J, MacDonald D, Schulte P (2010) Conservation genomics of Atlantic salmon: variation in gene expression between and within regions of the Bay of Fundy. Molecular Ecology, **19**, 1842-1859.

Whitehead A, Crawford DL (2006) Neutral and adaptive variation in gene expression. Proceeding of the National Academy of Sciences USA, **103**, 5425-5430.

Appendix A1 – Fold difference between immune challenged and control transcription states

Fold difference in gene transcription between control and immune challenged (Immune/Control) states for five Babine Lake tributary populations of juvenile rainbow trout. Tachek Creek not analyzed under immune challenged state due to insufficient quantities of RNA. Significant differences ($p < 0.05$) in transcription indicated by (*).

ID	Name	11 Mile	Cross	Duncan	Sutherland	Tsak
HEATH008	achainooligosaccharideslysozymerainbowtrout				0.76	
HEATH009	acidicRprotein.AY255630.1	0.76	1.6	1.51	1.02	1.26
HEATH012	AcylCoa_DHCA044583F2				3.25	
HEATH015	af223744transferrin				0.47	
HEATH017	AhR.AF065138.4	0.79	0.83	1.24	1.04	0.95
HEATH019	alcoholDH5	0.51*	1.16	0.95	0.94	0.83
HEATH022	aldehyde.DH7				0.97	
HEATH024	aldolase.EF042598.1				1.06	
HEATH025	aldolase.fructose-a				1.68	
HEATH028	Alpha-N-acetylgalactosaminidase[Salmosalar]		0.11		1	
HEATH031	aminesulfotransferase				0.49	
HEATH034	apocytochrome-b	1.28			1.03	0.58
HEATH035	apolipoprotein.a-i-1	1.05	0.49	0.3*	1.26	0.76
HEATH036	apolipoprotein.a-iv				0.48	
HEATH037	apolipoprotein.c-i	0.66	0.85	3.02	6.06	0.46
HEATH039	Arginase.AY056477.1				0.76	
HEATH040	argininеserine-richcoiled-coilprotein1		1.91*		0.87	
HEATH043	atpase6	0.6	0.66	1.06	1.62	
HEATH044	atpasesubunit6				1.8	
HEATH047	bbetapolypeptide				1.08	
HEATH048	beta_actin.FJ890357.1	2.07	1.27		0.48	0.89
HEATH049	beta-2microglobulintype2				0.85	
HEATH051	c-cmotifchemokine19precursor	0.87	1.15	3.96*	1.05	0.54
HEATH056	C3		1.08			
HEATH057	c3-like				4.34	
HEATH058	C3.U61753.F	1.1	0.77	0.51	0.8	0.84
HEATH059	C5.AF349001		0.86	0.51*	0.7	1.09
HEATH062	calcium.atpase.2				0.9	
HEATH063	calreticulinlike				1.27	
HEATH064	camp_dep_prot_kinase	0.97	0.81		0.84	0.86
HEATH065	carbonic.anhydrase.12				1.13	0.55
HEATH067	carboxymuconate-6-semialdehyde.decarboxylase	0.93	1.08	0.89	0.59	0.35
HEATH068	CAT.FJ226371.1				0.97	
HEATH069	Catalase.NM_001140302.1		1.62		6.81	

HEATH071	cathepsin_dU90321R				1.05	
HEATH072	cathepsin.h	0.54			1.06	0.94
HEATH073	cathepsin.l	0.96			1.15	0.98
HEATH074	cathepsin.l1	0.43	1.18	0.89	1.74	
HEATH076	cathepsin.y			0.71	0.86	0.82
HEATH079	CB_alternate.AB044939				0.63	
HEATH080	CBA.FJ226372.1	1.15	1.55	1.73	0.52	1.19
HEATH081	CbM60646				0.97	0.66
HEATH082	cellcyclecontrolprotein50a				1.07	
HEATH084	ceruloplasmin				0.71	
HEATH085	ceruloplasminisoformcra_b	0.88			1.19	2.02*
HEATH086	Ch.Y00716				1.99	
HEATH087	chemokine.13.precursor	0.75	0.65	0.85	1.17	0.99
HEATH088	chemokine.19				1.31	
HEATH091	cold-induciblerna-bindingprotein				1.02	
HEATH092	complementcomponentc8gammachainprecursor				3.03	
HEATH093	complementcomponentc9				0.2	
HEATH097	creatine.kinase	0.63	1.37		0.73	0.85
HEATH098	creatinekinaseb-type				0.61	0.58
HEATH099	Creatinekinases1				0.47	1.05
HEATH100	Creatinekinases2				1.71	
HEATH106	cyclinb2				0.76*	
HEATH107	cycling1	3.14	0.99		0.84	0.29
HEATH109	CYP1a1.AF015660.1				0.58	
HEATH113	CYP1c2.GU325709.F				0.89	1.26
HEATH114	cysteine.proteinase	0.49*	0.9		0.89	1.12
HEATH116	cytc.ox_subunit2	0.92	1.04	1.33	0.61	0.54
HEATH118	cytochrome.b-c1.complex	0.57	1.04		1.52	
HEATH119	cytochrome.c.ox.sub1					0.78
HEATH120	cytochromeb-c1	1.5			0.91	
HEATH121	cytochromep4502k4		0.82			
HEATH122	cytochromep450monooxygenasecyp2k1v2	1.04	0.86	0.75	0.39	0.72
HEATH123	cytox.EU524234.1	1.65			0.75	
HEATH126	dna-damage-inducibletranscript4protein				0.19	0.24
HEATH128	ecto-adp-ribosyltransferase				0.95	
HEATH129	ecto-adp-ribosyltransferase5precursor	0.86			1	0.97
HEATH130	EF1a.AF498320	1.37	0.89	0.76	1.16	1.6
HEATH131	EF1delta				1.11	
HEATH132	elastase_inhibitorDQ908922.1	0.41	1.1	0.21*	0.77	3.46*
HEATH133	elastase-1	0.7*			1.15	1.2
HEATH134	elastase-like.serine.protease	0.86			0.89	0.84
HEATH136	elongationfactor2	0.37			1.38	
HEATH137	enolase.3-2				1.08	
HEATH139	ependymin				0.99	0.95
HEATH141	ependymin.precursor	0.89			0.83	1.07
HEATH143	eukaryotictranslationinitiationfactorsubunit2		0.93	1.61	0.97	

HEATH144	F26BP.EG879358	1.04			0.75	1.5
HEATH145	FABP.U95296	0.89	1.23	0.86	0.69	1.44
HEATH146	FADD[Salmosalar]				0.94	
HEATH149	ferritin.FJ890362.1	0.5	0.95	0.57	0.99	2.05
HEATH150	fibrinogen.alpha.chain				0.4	
HEATH152	fibrinogen.gamma				5.38	
HEATH153	fishvirusinducedtrimprotein	0.78	2.42*	0.89	0.78	0.57*
HEATH154	fission.process.protein1				1.4	
HEATH157	G6P.isomerase	0.36			1.3	
HEATH160	GAPDH2			0.92	0.89	
HEATH162	GLUT1.AF247728.F				0.73	
HEATH169	Glycogen_debranchingDY731517				0.59	
HEATH174	GR1.Z54210				0.03	
HEATH177	growthfactorbindingprotein.JF920120.1				1.49*	
HEATH181	h2a.histone				1.35	
HEATH183	Hemopexin.AF281339.1.F				1.89	
HEATH184	hepcidin-1.precursor				3.66	
HEATH185	Hepcidin.AF281354.1.R2	0.62			1.33	0.27
HEATH187	Hsc70	0.79	1.38	1.44	1.17	0.83
HEATH188	Hsc70b.AB196461.F2	0.51	1.23		0.41	2.3*
HEATH191	HSP27_var1.AB255361				0.93	
HEATH192	hsp30.U19370.1	1.28	0.59		0.66	0.81
HEATH193	Hsp47.AB196463				0.98	0.86
HEATH197	Hsp90Ba.AB196457	0.62			1.02	
HEATH198	Hsp90Bb.AB196458	0.82	1.07		0.8	0.79
HEATH199	hyperosmoticglycinerichprotein	0.8	1.18	2.16	0.93	1.03
HEATH200	IGF1.M95183				0.68	
HEATH201	IGF2.M95184	1.04			0.67	0.29
HEATH202	IGFbind3.HM536183.1	0.58	0.16	1.11	1.13	
HEATH203	IGFbind5.HM536184.1				0.42	2.62
HEATH205	IL-1.DQ778946				0.89	1.6
HEATH207	IL10b.FR691804.1				1.1	
HEATH210	IL2.AM422779	0.58*	0.62*	0.22*	0.99	0.89
HEATH212	IL8.DQ778949				1.13*	
HEATH215	inositol.hexakisphosphate.kinase1				1.17	6.13
HEATH216	Insulin_receptor_a.AF062496				1.25	0.88
HEATH217	Insulin_receptor_c.AF062498.F1	0.52			0.98	0.76*
HEATH220	interleukin-1.FJ890361.1				0.82	
HEATH225	liver-basic.fatty.acid.binding.protein.a				4.96	
HEATH226	lysozymeg		0.82			
HEATH228	MCH1.M25754.1				0.92	
HEATH231	metalloreductasesteap4	1.64			0.89	0.74
HEATH232	MHC1.AF296359				0.47	
HEATH233	MHC2.AF296390	0.89	1.61	1.11	0.88	0.87
HEATH234	MHCIalpha3.U80312.1	0.59	1.32	0.54*	0.75	1.69*
HEATH235	MHCIantigen.AF091780.1		1.09		1.26	1.34

HEATH236	MHCIantigen.AF162871.1				1.01	
HEATH237	MHCII.EF432124.1				1.11	
HEATH240	mitogen-activatedproteinkinaseorganizer1		0.41			
HEATH243	Mx2.U47945	0.45*		0.61*	0.83	1.58
HEATH246	nadhDHsub2				1.04	1.26
HEATH247	nadhDHtype2				1.03	
HEATH248	NaKATPase1ab.AY319390	0.84	0.59		0.97	
HEATH249	nicotinamideribosidekinase2				0.86	1.19
HEATH250	nramp-a.EF042597.1				0.52	0.79
HEATH252	orc1lprotein				0.9	
HEATH257	Peroredoxin.U27125				1.08	
HEATH262	pigmentepithelium-derivedfactor		0.32			
HEATH263	plastin-2-partial	2.44			1.78	
HEATH266	PRLII.S66606.1				0.9	
HEATH267	profilin-1	0.62	1.23	1.87	1.08	0.82
HEATH268	profilin-1isoform1	0.74	1.01		0.58	
HEATH269	profilin.2	0.72			1.23	
HEATH272	proteasomeactivatorcomplexsubunit2				2.11	1.22
HEATH273	proteasomesubunitalphatype-6	0.28*	2.35		0.56	1.26
HEATH276	Pyruvate_kinaseDW582027	0.4	1.2		1.6	
HEATH279	retinol-binding				1.04	
HEATH280	ribulose-phosphate3-epimerase	2.91			1.04	
HEATH281	ribunucleoproteinL-like.DQ914957.1				0.88	
HEATH282	rikencdnaisoformcra_a		0.99		0.51	0.76
HEATH283	rpL8.FJ226373.1	0.57	1.36	1.05	1.25	1.78
HEATH284	S100-A11[Salmosalar]	1.03	0.69	0.28*	0.24	0.82
HEATH286	sec24aprotein	1.39	0.66*		0.81	1.43
HEATH289	Na/K ATPase subunitalpha-1precursor		0.88			
HEATH291	solute.carrier.25-2				0.84*	
HEATH292	somatolactin_precursor		1.15		0.83	
HEATH294	Stathmin[Salmosalar]				0.83	
HEATH296	SAA				5.98	
HEATH299	Syntaxin-7				0.46	
HEATH300	t-complex polypeptide1				1.49*	
HEATH303	tc1-liketransporase	0.75	1.04	1.39	0.87	0.42
HEATH307	TLR1	1.33	0.54	1.45	0.52	1.07
HEATH308	TLR5	0.8	0.47		0.74	0.69
HEATH312	TLR9	0.78			0.7	0.53*
HEATH313	TNFa				0.48	
HEATH314	TNFreceptor	0.52			0.98	0.35
HEATH315	transcriptioninitiationfactortfiidsubunit4b-like	0.87			1.04	
HEATH317	translation.initiation.factor.4e		0.95		1.29	
HEATH318	translation.initiation.factor.6				0.85	0.94
HEATH319	translation.initiation.factor2		1.16		0.82	
HEATH321	transportproteinsec61subunitgamma	0.62	1.4	1.2	0.57	2.29
HEATH323	tsuppressor_p53.AF223818.1				0.48	0.45

HEATH327	Ubiquitin.AB036060.F1	1.06	1.34	1.95	0.83	1.15
HEATH329	Uracil_DNA_glycosylseCA041722		0.91		0.8	0.96
HEATH335	insulin-like_growth_factor-binding_protein_2b	0.47*	0.62		1.12	2.1*
HEATH336	insulin-like_growth_factor-binding_protein_2a	4.17	0.71		1.22	0.39
HEATH339	ribosomal_protein_L8_(rpL8)	0.84	1.4	1.38	0.76	
HEATH340	estrogen_receptor_alpha_(ER_alpha)				0.69	1.71*
HEATH343	natural_killer_cell_enhancement_factor_(Nkef)	0.87	0.86	1.58	1.03	1.66
HEATH347	gonadotropin_alpha_subunit				0.95	0.41
HEATH349	virus-inducible_stress_protein_(VISP)				0.23	
HEATH352	growth_hormone_2	1.86			1.15	1.13
HEATH355	CLOCK1b_(Clock1b)	0.7	0.97	0.73*	0.86	0.68
HEATH356	CLOCK1a_(Clock1a)				0.96	1.23
HEATH357	annexin_mRNA	2.1*			1.55	0.99
HEATH363	complement_factor_D	0.87			0.76	
HEATH365	lag-3	0.74			0.67	1.14
HEATH366	csf-3				0.55	
HEATH369	thymosin				0.96	0.63
HEATH371	mrap2				1.1	

Appendix A2 – Fold difference between temperature challenged and control transcription states

Fold difference in gene transcription between control and temperature challenged states for six Babine Lake tributary populations of juvenile rainbow trout. Significant differences ($p < 0.05$) in transcription indicated by (*).

ID	Name	11 Mile	Cross	Duncan	Sutherland	Tachek	Tsak
HEATH008	achainooligosaccharideslysozymerainbowtrout	0.73		1.44	1		
HEATH009	acidicRprotein.AY255630.1	0.42	0.52	0.62	2.05	2.17*	1.67
HEATH015	af223744transferrin				0.14		
HEATH017	AhR.AF065138.4	0.86	1.14	0.65	0.88	0.75	1.44
HEATH019	alcoholDH5	0.94	0.6	0.73	1.97	1.54	0.51
HEATH022	aldehyde.DH7	0.93					
HEATH024	aldolase.EF042598.1				4.68		
HEATH028	Alpha-N-acetylgalactosaminidase[Salmosalar]		0.07	1.04		4.33	
HEATH031	aminesulfotransferase	1.37		0.74			
HEATH034	apocytochrome-b	0.79	1.09	1.46	1.06	1.4	0.6
HEATH035	apolipoprotein.a-i-1	1.67	1.41	0.46	1.21	0.59	1.17
HEATH036	apolipoprotein.a-iv	1.01					
HEATH037	apolipoprotein.c-i	0.56*	4.15	1.46	3.49	1.63	0.6
HEATH040	argininеserine-richcoiled-coilprotein1				3.45	1.92	
HEATH043	atpase6	0.55	0.27*	0.99	2.83		
HEATH044	atpasesubunit6		1.3			1.62*	
HEATH047	bbetapolypeptide	1.55			0.64*		
HEATH048	beta_actin.FJ890357.1	1.15			1.2	1.93	0.33
HEATH049	beta-2microglobulintype2	0.87					
HEATH051	c-cmotifchemokine19precursor	0.89	0.62	1.69	1.64	2.2	0.16
HEATH058	C3.U61753.F	1.8*	1.39	1	0.97	0.76	0.89
HEATH059	C5.AF349001	0.74*	0.82	0.56*	0.81	1.04	0.85
HEATH062	calcium.atpase.2	1.19			1.02		
HEATH063	calreticulinlike				1.05		
HEATH064	camp_dep_prot_kinase	1.02	0.76	1.75*	0.67*	1.19	0.72
HEATH065	carbonic.anhydrase.12	1.37		0.72	1.35	2*	0.51
HEATH067	carboxymuconate-6-semialdehyde.decarboxylase	0.96	0.59	0.94	1.07	4.64	0.25
HEATH068	CAT.FJ226371.1	0.93					
HEATH069	Catalase.NM_001140302.1	0.86			1.82		
HEATH071	cathepsin_dU90321R	0.81			1.46		
HEATH072	cathepsin.h	0.61	0.81	1.3	0.97		1.2
HEATH073	cathepsin.l	1.06	1.38*	0.96	0.77		1.26
HEATH074	cathepsin.l1	0.39	2.79	0.8	3.77*	2.86	
HEATH076	cathepsin.y	0.96	1	0.86	0.77*	0.96	0.84
HEATH079	CB_alternate.AB044939	1.16					
HEATH080	CBA.FJ226372.1	0.46	0.28	1.45	1.96	2.75	0.31
HEATH081	CbM60646	0.86		0.74	1.27*	1.53	0.57
HEATH083	cellular.retinoic.acid-binding.protein.1	0.94					
HEATH085	ceruloplasminisoformcra_b	0.55			1.26		1.18
HEATH087	chemokine.13.precursor	1.08	0.79	0.66*	1.35*	1.05	1.27
HEATH088	chemokine.19				1.75		
HEATH097	creatine.kinase	0.55	0.63	1.46	1.45	0.84	0.6
HEATH098	creatinekinaseb-type	1.05		0.9	0.65	1.71*	0.53
HEATH099	Creatinekinases1	0.95			1	4.97	
HEATH100	Creatinekinases2	0.39			2.55	1.34	
HEATH107	cycling1	0.94	0.79	0.2	0.71*	1.1	0.87
HEATH109	CYP1a1.AF015660.1	0.44			0.61	3.3*	
HEATH113	CYP1c2.GU325709.F	0.95	1.12	0.68*	1	1.43	1.52
HEATH114	cysteine.proteinase	0.31*			2.37	1.53	
HEATH115	cysteineandglycine-richprotein1	0.9					
HEATH116	cytc.ox_subunit2	0.43	0.56	0.75	0.9	1.29	0.33
HEATH118	cytochrome.b-c1.complex	0.55	0.68	0.98	1.47	1.61*	
HEATH119	cytochrome.c.ox.sub1					2.46*	0.98
HEATH120	cytochromeb-c1	1.22			0.84	2.67*	

HEATH122	cytochromep450monooxygenasecyp2k1v2	1.45	0.63	1.11	0.57	0.73	0.65
HEATH123	cytox.EU524234.1	0.79			0.74		1.12
HEATH126	dna-damage-inducibletranscript4protein	1.76*	1.22	1.08	0.11	0.86	0.21
HEATH129	ecto-adp-ribosyltransferase5precursor	1.06		0.76	1.2	1.18	0.87
HEATH130	EF1a.AF498320	0.61	1.25	1.03	1.34	0.95	0.99
HEATH131	EF1delta				1.69*	3.51	
HEATH132	elastase_inhibitorDQ908922.1	0.58	2.23	0.72	0.77	0.61	1.54
HEATH133	elastase-1	0.89		1.96	1.33	0.94	1.03
HEATH134	elastase-like.serine.protease	1.03		0.83*	0.82	1.4	0.83
HEATH136	elongationfactor2	0.54	0.51	1.12	0.84		
HEATH138	enolase2					18.72*	
HEATH139	ependymin	0.89			1.08		
HEATH141	ependymin.precursor	0.91			1.11	1.41	0.96
HEATH143	eukaryotictranslationinitiationfactorsubunit2	0.68	0.73	0.78	0.96		
HEATH144	F26BP.EG879358	0.8		2.12	0.83	1.58	1.38
HEATH145	FABP.U95296	0.83	0.87	0.63	0.77	2.21*	1.67
HEATH146	FADD[Salmosalar]	1.17	0.4				
HEATH149	ferritin.FJ890362.1	0.82	1.48	0.49	1.74	0.43*	2.31
HEATH150	fibrinogen.alpha.chain	1.01					
HEATH153	fishvirusinducedtrimprotein	0.49	0.97	0.97	2.75	0.65	1.43
HEATH154	fission.process.protein1					11.41	
HEATH157	G6P.isomerase				1.54		
HEATH160	GAPDH2	0.2	4.68	5.77	1.06	5.99	
HEATH162	GLUT1.AF247728.F	0.98			1.01	1.8*	
HEATH168	glycerol-3-phosphateDH			1.49*			
HEATH185	Hepcidin.AF281354.1.R2	0.62	0.65	1.25	1.1	2.18*	0.27
HEATH186	highchoriolyticenzyme1precursor			1.49			
HEATH187	Hsc70	0.58	0.61	1.12	1.69	0.83	0.65
HEATH188	Hsc70b.AB196461.F2	0.45	0.71		0.71	2.73	0.36*
HEATH191	HSP27_var1.AB255361	0.68			0.7	2.23	0.94
HEATH192	hsp30.U19370.1	3.12	2.45	0.36	0.69	0.7	1.9
HEATH193	Hsp47.AB196463	0.84		0.57	1.05	1.05	0.77
HEATH197	Hsp90Ba.AB196457	0.44*			1.57		
HEATH198	Hsp90Bb.AB196458	0.64	0.31*		1.28	1.2	0.58
HEATH199	hyperosmoticglycinerichprotein	0.73	0.27*	1.15	3.25	1.21	0.5
HEATH201	IGF2.M95184	1.18		1.28	0.6	2.91	0.32
HEATH202	IGFbind3.HM536183.1	0.65	0.12	0.74	1.53	2.23	
HEATH203	IGFbind5.HM536184.1	0.95		2.08	0.46		1.09
HEATH205	IL-1.DQ778946	0.91	0.48		1.24	1.96*	1.08
HEATH207	IL10b.FR691804.1	1.23			0.96		
HEATH210	IL2.AM422779	0.97	1.16	0.53*	1.08	0.62*	1.02
HEATH215	inositol.hexakisphosphate.kinase1	1.12			1.51		1.51
HEATH216	Insulin_receptor_a.AF062496	1.56*		1.39			1.57
HEATH217	Insulin_receptor_c.AF062498.F1	0.73		1.06	0.91	1.16	0.99
HEATH225	liver-basic.fatty.acid.binding.protein.a	0.84					
HEATH226	lysozymeg			1.09		2.39	
HEATH231	metalloreductasesteap4	1.38			1.04	1.15	0.85
HEATH232	MHC1.AF296359				0.81		
HEATH233	MHC2.AF296390	0.87	0.69*	0.67	0.88	1.28	1.05
HEATH234	MHCIalpha3.U80312.1	0.79	1.39	1.05	0.74	1.02	1.01
HEATH235	MHCIantigen.AF091780.1	1.06	0.71	0.79	1.21		
HEATH236	MHCIantigen.AF162871.1					1.38	
HEATH237	MHCII.EF432124.1				1.35		
HEATH243	Mx2.U47945	0.92		0.75	1.3		1.16
HEATH246	nadhDHsub2	1.16		1.09	1.14	1.4	1.33
HEATH247	nadhDHtype2	1.58					
HEATH248	NaKATPase1ab.AY319390	0.65			1		
HEATH249	nicotinamideribosidekinase2	1.1	0.51	0.64*	1.84	1.77	0.96
HEATH250	nramp-a.EF042597.1	2.32		1.75	0.41		0.61
HEATH257	Peroredoxin.U27125				1.16		
HEATH263	plastin-2-partial	1.6			2.05		
HEATH266	PRLII.S66606.1		0.92	1.03	2.02*	1.66	
HEATH267	profilin-1	0.4	0.4*	1.09	1.42	1.48	0.75
HEATH268	profilin-1isoform1	0.47			0.83	2.68*	

HEATH269	profilin.2	0.48		0.88	1.56*	1.02	
HEATH272	proteasomeactivatorcomplexsubunit2			1.89	0.84		1.09
HEATH273	proteasomesubunitalphatype-6	0.56*	2.41	0.51*	0.73	0.73	1.07
HEATH276	Pyruvate_kinaseDW582027	0.33*			2.06*		
HEATH280	ribulose-phosphate3-epimerase	1.05			0.19		
HEATH282	rikencdnaisoformcra_a	1.38	1.07	0.97	0.62	1.47	2.79
HEATH283	rpL8.FJ226373.1	0.23	0.39		2.21*	2.28	1.12
HEATH284	S100-A11[Salmosalar]	1.61	1.76	0.91	0.28	0.72	1.07
HEATH286	sec24aprotein	1.85	0.64*	1.52	0.51*	0.89	2.02
HEATH289	Na/K ATPase subunitalpha-1precursor	0.68	5.42				
HEATH292	somatolactin_precursor.AF223890.1GI:8895689				1.44		
HEATH294	Stathmin[Salmosalar]	1.07					
HEATH303	tc1-liketransporase	0.84	0.25	0.96	1.02	2.53	0.25
HEATH307	TLR1.NM_001166101	1.54	0.22*	1.24	0.52	0.79	1.36
HEATH308	TLR5.AB091105	0.77	0.22	0.87	0.66	1.44	0.4*
HEATH312	TLR9.NM_001129991	1.12	0.91	0.97	0.63*	1.25*	0.71
HEATH314	TNFreceptor	0.73	0.46*	0.88	1.09	1.75*	0.16
HEATH315	transcriptioninitiationfactortfiidsubunit4b-like	0.95			1.35		
HEATH317	translation.initiation.factor.4e	0.86	0.42		2.61		
HEATH318	translation.initiation.factor.6						0.75
HEATH319	translation.initiation.factor2	0.23				2.46	
HEATH321	transportproteinsec61subunitgamma	0.77	0.68	1.09	1.93	0.57	1.92
HEATH323	tsuppressor_p53.AF223818.1	2.57			0.27	1.77	0.33
HEATH324	tubulin.alpha-8	1.02			0.58		
HEATH327	Ubiquitin.AB036060.F1	0.78	0.32	1.06	1.43	1.2	0.79
HEATH329	Uracil_DNA_glycosylseCA041722	0.44	0.45	0.37	0.55	2.56*	0.76
HEATH335	insulin-like_growth_factor-binding_protein_2b	0.66*	1.79	2.23	0.93	1.01	1.52
HEATH336	insulin-like_growth_factor-binding_protein_2a	1.44	0.83	1.18	0.53	1.02	0.55
HEATH339	ribosomal_protein_L8_(rpL8)	0.75	0.57	0.99	1.48	1.13	
HEATH340	estrogen_receptor_alpha_(ER_alpha)	1.02			0.8		1.07
HEATH343	natural_killer_cell_enhancement_factor_(Nkef)	0.59	0.47	1.23	1.67		0.99
HEATH347	gonadotropin_alpha_subunit	0.56			0.96	2.19	0.4
HEATH352	growth_hormone_2	1.59		0.76	1.08		1.18
HEATH355	CLOCK1b_(Clock1b)	0.96	0.8	0.76	0.87	0.75	0.7
HEATH356	CLOCK1a_(Clock1a)	1.15	0.33	0.64		1.97*	0.97
HEATH357	annexin	1			2.21		1.48
HEATH363	complement_factor_D	1.21			0.84		
HEATH365	lag-3	0.9		0.93	0.55	0.89	1.36*
HEATH366	csf-3					3.29	
HEATH369	thymosin				1.78*		1.45
HEATH371	mrap2	0.78					

Appendix B – Phenotypic divergence estimates (P_{ST}) for genes

Phenotypic divergence estimates (P_{ST}) based on transcription of genes at resting state (Control P_{ST}) temperature challenged (Temp P_{ST}) and immune challenged transcription (Path P_{ST}) as well as putative function based on analysis with BLAST2GO software.

ID	Name	Control P_{ST}	Temp P_{ST}	Path P_{ST}	Putative Function
HEATH008	achainooligosaccharideslysozymerainbowtrout		0.14		response to stress
HEATH009	acidicRprotein.AY255630.1	0.11	0.19	0.18	cellular process
HEATH017	AhR.AF065138.4	0.16	0.23	0.27	response to stimulus
HEATH019	alcoholDH5	0.16	0.23	0.28	metabolic process
HEATH028	Alpha-N-acetylgalactosaminidase[Salmosalar]		0.15		metabolic process
HEATH034	apocytochrome-b	0.25	0.20		metabolic process
HEATH035	apolipoprotein.a-i-1	0.16	0.21	0.23	metabolic process
HEATH037	apolipoprotein.c-i	0.23	0.19	0.18	metabolic process
HEATH040	argininеserine-richcoiled-coilprotein1	0.10		0.22	metabolic process
HEATH043	atpase6	0.19	0.19	0.24	metabolic process
HEATH048	beta_actin.FJ890357.1	0.09	0.11	0.19	cellular process
HEATH051	c-cmotifchemokine19precursor	0.10	0.16	0.18	immune response
HEATH058	C3.U61753.F	0.20	0.11	0.16	immune response
HEATH059	C5.AF349001	0.16	0.11		immune response
HEATH064	camp_dep_prot_kinase	0.20	0.17		cellular process
HEATH065	carbonic.anhydrase.12	0.26	0.15		metabolic process
HEATH067	carboxymuconate-6-semialdehyde.decarboxylase	0.24	0.27	0.15	metabolic process
HEATH072	cathepsin.h	0.13	0.24		metabolic process
HEATH073	cathepsin.l	0.19	0.19		metabolic process
HEATH074	cathepsin.l1	0.09	0.10	0.18	metabolic process
HEATH076	cathepsin.y	0.16	0.06		metabolic process
HEATH080	CBA.FJ226372.1	0.10	0.21	0.18	cellular process
HEATH081	CbM60646	0.19	0.13		immune response
HEATH085	ceruloplasminisoformcra_b	0.29	0.28		metabolic process
HEATH087	chemokine.13.precursor	0.17	0.17	0.07	immune response
HEATH097	creatine.kinase	0.10	0.27	0.29	metabolic process
HEATH098	creatinekinaseb-type		0.10		metabolic process
HEATH100	Creatinekinases2		0.23		metabolic process
HEATH107	cycling1	0.03	0.07	0.04	response to stress
HEATH113	CYP1c2.GU325709.F	0.21	0.10		metabolic process
HEATH114	cysteine.proteinase	0.18	0.24	0.19	metabolic process
HEATH116	cytc.ox_subunit2	0.08	0.18	0.17	metabolic process
HEATH118	cytochrome.b-c1.complex		0.21		metabolic process
HEATH120	cytochromeb-c1		0.24		metabolic process
HEATH122	cytochromep450monooxygenasecyp2k1v2	0.18	0.18	0.06	metabolic process
HEATH126	dna-damage-inducibletranscript4protein	0.08	0.13		response to stress
HEATH129	ecto-adp-ribosyltransferase5precursor	0.19	0.06		metabolic process
HEATH130	EF1a.AF498320	0.10	0.17	0.06	cellular process
HEATH132	elastase_inhibitorDQ908922.1	0.27	0.20	0.26	metabolic process
HEATH133	elastase-1	0.26	0.14		response to stress
HEATH134	elastase-like.serine.protease		0.16		metabolic process
HEATH141	ependymin.precursor		0.16	0.25	cellular process
HEATH143	eukaryotictranslationinitiationfactorsubunit2	0.16	0.04		cellular process
HEATH144	F26BP.EG879358	0.15	0.26		metabolic process
HEATH145	FABP.U95296	0.16	0.08	0.07	metabolic process
HEATH149	ferritin.FJ890362.1	0.25	0.24	0.18	metabolic process

HEATH153	fishvirusinducedtrimprotein	0.06	0.11	0.27	immune response
HEATH160	GAPDH2		0.04		metabolic process
HEATH162	GLUT1.AF247728.F		0.14		metabolic process
HEATH185	Hepcidin.AF281354.1.R2	0.19	0.08		response to stress
HEATH187	Hsc70	0.05	0.11	0.16	response to stress
HEATH188	Hsc70b.AB196461.F2	0.16	0.15	0.18	response to stress
HEATH191	HSP27_var1.AB255361	0.17	0.25		response to stress
HEATH192	hsp30.U19370.1	0.05	0.08		response to stress
HEATH193	Hsp47.AB196463	0.24	0.11		response to stress
HEATH197	Hsp90Ba.AB196457	0.21			response to stress
HEATH198	Hsp90Bb.AB196458	0.17	0.20	0.20	response to stress
HEATH199	hyperosmoticglycinerichprotein	0.08	0.21	0.15	cellular process
HEATH201	IGF2.M95184	0.23	0.20		growth
HEATH202	IGFbind3.HM536183.1	0.25	0.11	0.10	growth
HEATH203	IGFbind5.HM536184.1	0.18	0.16		growth
HEATH205	IL-1.DQ778946	0.20			immune response
HEATH210	IL2.AM422779	0.25	0.17	0.22	immune response
HEATH215	inositol.hexakisphosphate.kinase1	0.25	0.22		metabolic process
HEATH216	Insulin_receptor_a.AF062496		0.04		growth
HEATH217	Insulin_receptor_c.AF062498.F1	0.08	0.13		growth
HEATH231	metalloreductasesteap4		0.23		cellular process
HEATH233	MHC2.AF296390	0.13	0.11	0.19	immune response
HEATH234	MHCIalpha3.U80312.1	0.12	0.08	0.27	immune response
HEATH235	MHCIantigen.AF091780.1	0.21			immune response
HEATH243	Mx2.U47945	0.27	0.15	0.20	immune response
HEATH246	nadhDHsub2	0.21	0.03		metabolic process
HEATH248	NaKATPase1ab.AY319390	0.13		0.14	metabolic process
HEATH249	nicotinamideribosidekinase2	0.25	0.08		metabolic process
HEATH250	nramp-a.EF042597.1		0.21		response to stimulus
HEATH263	plastin-2-partial		0.20		immune response
HEATH266	PRLII.S66606.1		0.10		metabolic process
HEATH267	profilin-1	0.11	0.17	0.15	cellular process
HEATH268	profilin-1isoform1	0.16	0.18	0.15	cellular process
HEATH269	profilin.2		0.18		cellular process
HEATH272	proteasomeactivatorcomplexsubunit2		0.12		response to stress
HEATH273	proteasomesubunitalphatype-6	0.30	0.18		response to stress
HEATH276	Pyruvate_kinaseDW582027			0.10	metabolic process
HEATH280	ribulose-phosphate3-epimerase		0.12		metabolic process
HEATH282	rikencdnaisoformcra_a	0.08	0.13		cellular process
HEATH283	rpL8.FJ226373.1	0.15	0.23	0.16	cellular process
HEATH284	S100-A11[Salmosalar]	0.26	0.05	0.23	response to stimulus
HEATH286	sec24aprotein	0.25	0.08	0.11	cellular process
HEATH303	tc1-liketransporase	0.07	0.25	0.16	metabolic process
HEATH307	TLR1.NM_001166101	0.26	0.18	0.11	immune response
HEATH308	TLR5.AB091105	0.23	0.30	0.17	immune response
HEATH312	TLR9.NM_001129991	0.25	0.15		immune response
HEATH314	TNFreceptor	0.07	0.21		immune response
HEATH321	transportproteinsec61subunitgamma	0.26	0.22	0.10	cellular process
HEATH323	tsuppressor_p53.AF223818.1		0.25		response to stress
HEATH327	Ubiquitin.AB036060.F1	0.05	0.18	0.23	response to stress
HEATH329	Uracil_DNA_glycosylseCA041722	0.17	0.27		response to stress
HEATH335	insulin-like_growth_factor-binding_protein_2b	0.24	0.26		growth
HEATH336	insulin-like_growth_factor-binding_protein_2a	0.12	0.07		growth
HEATH339	ribosomal_protein_L8_(rpL8)	0.08	0.15	0.23	cellular process

HEATH340	estrogen_receptor_alpha_(ER_alpha)		0.18		growth
HEATH343	natural_killer_cell_enhancement_factor_(Nkef)	0.15	0.14	0.23	immune response
HEATH352	growth_hormone_2_gene,_intron_C	0.16	0.04		growth
HEATH355	CLOCK1b_(Clock1b)_mRNA,_partialsscds	0.03	0.08	0.04	cellular process
HEATH356	CLOCK1a_(Clock1a)_mRNA,_partialsscds	0.13	0.14		cellular process
HEATH357	annexin_mRNA,_completesscds	0.21	0.06		immune response
HEATH365	lag-3	0.11	0.11		immune response

VITA AUCTORIS

Kyle Wellband was born in Bridgewater, Nova Scotia in 1986. He grew up in Sackville, New Brunswick where he developed a passion for science while volunteering at the Tantramar Wetlands Centre. After a brief stint at the University of Victoria he completed his Bachelor's of Science degree with Honours in Biology studying genome size variation in amphipods at Mount Allison University in 2008. He then found his way to Windsor, Ontario where he studied local adaptation in Babine Lake rainbow trout with Dr. Daniel Heath at the Great Lakes Institute for Environmental Research.